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A MODEL SYSTEM FOR TESTING THE MICROBIOLOGICAL STABILITY OF FOODS PROCESSED IN LAMINATED FLEXIBLE POUCHES

by

LORRAINE M. RAUHALA 

A THESIS

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled ..A Model System for.... .Testing the Microbiological Stability of Foods Processed. .in Laminated Flexible Pouches..... submitted by ....Lorraine M. Rauhala..... in partial fulfilment of the requirements for the degree of Master of Science.....

ABSTRACT

To create a model system for determining the microbiological stability of food processed in flexible pouches, B. stearothermophilus was used as a test micro-organism to determine a suitable time-temperature treatment for retorting vacuum packaged small potatoes in a water cook process. A steam retort was modified to enable a water cook process with an air overpressure of c. 68.95 k Pa (10 lb/in^2) to be used for this purpose.

The D and z values of the test micro-organism were determined using the retort as a laboratory tool. This was done after inoculating the test micro-organism (B. stearothermophilus) on the surface of potatoes in plastic pouches which were then evacuated and heat sealed. The D and z values determined by this practical method were confirmed by heat resistant tests of the micro-organism in glass ampoules in an oil bath, at a higher temperature than was attainable with this retort.

A suitable time-temperature process which did not overcook the potatoes yet yielded a satisfactory sporicidal treatment was found to be 20 minutes at 121.1°C.

Proof that this process was satisfactory was obtained by processing pouches of potatoes bearing the natural level of contaminating spores (determined to be c. 4 x 10^3/pouch) at different times at 121.1°C and incubating at 30°C for 6 months. All experimental pouches heated for 17 up to 25 minutes showed no evidence of microbial growth after 6 months of incubation.

It is concluded that such a system of testing could be used for any type of food if the natural level of microbial contamination is

determined for the raw product and related to the heat resistance of a suitable test micro-organism.

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k Pa	kilo Pascal, unit of pressure in the metric system, where a Pascal is $kg/m\ sec^2$
F	the number of minutes required to destroy a given number of spores or vegetative cells of a particular organism at a given temperature
F_o	the number of minutes required to destroy a specified number of spores at 121.1°C (250°F) when z = 10°C (18°F)
F	the critical F value determined from a statistical analysis of the data and its significance as determined at the α level
θ	time
T	temperature
N	number of colonies/ml

I. INTRODUCTION

Preservation of food has long been a major concern to man. It was not until the development of the hermetically sealed glass jars and tinned cannisters in the early 1800's that man was able to achieve a shelf life of years for perishable products.

With the introduction of plastics, it was inevitable that uses would be found for them in the food preservation industry. Initially, the use of food containers made of plastic materials was restricted to dehydrated, frozen or refrigerated products with high sugar, salt or high acid content, which inhibited but did not necessarily destroy the micro-organisms present. The methods used to package such semi-preserved foods can be expensive and this procedure does not eliminate the potential for microbial growth if these foods are not kept refrigerated or frozen.

With the development of newer plastic film laminations that could withstand retort temperatures and restrict the passage of gases, and with the development of new heat processing techniques the production of foods in flexible pouches with a comparable shelf life to foods packaged in cans and glass jars was possible. With this development it was possible to apply shorter heating times than was possible for a rigid cylindrical container holding an equal volume of product and processed at the same temperature. The shorter processing time offered the advantage of reducing the degree of destruction of heat sensitive constituents of the food and of minimizing overcooking of the outer

layers of the pack especially when the product is heated by conduction. This quality advantage is attained by the more rapid rate of heat transfer into the food in the flexible package because of its thinner cross-sectional dimension.

So far no simple reliable method of determining the microbiological efficiency of heat treatment with flexible packages using water with a superimposed air pressure rather than a homogeneous mixture of steam and air, has been published. Such heat treatments would vary according to the type of food processed to make it microbiologically stable and acceptable. The heat treatments may also vary according to variety, maturity, etc. of various fruits and vegetables.

An attempt has been made to set up a test procedure for small whole potatoes in flexible pouches which may be used as a model for other types of non-acid food. This procedure involves testing pouches of potatoes to determine if they are microbiologically stable and meet the generally accepted level of heat processing (a 12 D process) for food with a pH value above 4.5; the D value being the time necessary for 90% destruction of micro-organisms at any given constant temperature. If the natural level of bacterial spore contamination for a given product is assessed together with the heat resistance of the predominant micro-organism, then the process necessary to yield a 12 D treatment can be determined. For example, if the natural contamination is 10^3 resistant spores/pack a 12 D treatment would yield one spore or the potential of one spoiled pack in 10^9 packs. To verify that such a degree of spoilage existed by incubating processed samples would be impossible on a laboratory scale. However, the degree of spoilage could be determined by using a heat-resistant spore-forming bacterial culture which could

be used as a test organism for any food and, by comparing the heat resistance and numbers of naturally occurring contaminating spores, make a calculation of the heat-treatment required for any food. The most widely used heat-resistant organisms for non-acid foods is P.A. 3679 (Clostridium sporogenes). A standardized inoculum greater than the naturally occurring contamination, for example, 10^4 spores/pouch, could be used in pouches undergoing processing at a given temperature with various treatment times so as to achieve different levels of spoilage. The efficiency of the treatment would be assessed by determining the number of surviving spores/pouch after treatment. By determining the D value of the reference organism under such conditions it would be possible to predict the time and temperature required for a 12 D treatment. Once the 12 D treatment had been determined for the test organism this could be converted to the time-temperature treatment required to destroy the naturally occurring contaminating spores in the food under study.

II. LITERATURE REVIEW

A. Characteristics and Properties of Plastic Films

In order that the flexible pouch can be used for shelf-stable foods it must meet the following requirements:

1. ability to withstand thermal processing at temperatures greater than 100°C and remain appreciably unaltered in appearance and strength;
2. capability of providing adequate protection for the food product during packaging, retorting, merchandising and storage;
3. low gas permeability, especially to oxygen;
4. low moisture permeability;
5. economy;
6. heat sealability;
7. resistance to fat, oils, etc.;
8. dimensional stability;
9. chemical inertness, imparting no taste to food;
10. resistance to tearing, abrasion, pinholing;
11. suitability for automatic processing;
12. occlusion of light, especially for light sensitive foods;
13. resistance to penetration by micro-organisms;
14. absence of transference of any toxic substances to the food from the laminated film.

Many people have studied various flexible films to determine if they met the desirable properties and requirements listed above.

Hu et al. (1955) stated that there were then only seven films that could withstand heat processing at 121.1°C (250°F). These were Saran, Cellophane, Trithene, Teflon, polyvinyl chloride film for blood packs, Tygon S22.2 and Mylar polyester. Cellophane was undesirable because it deteriorated physically at 121.1°C in 30 minutes. Tygon, polyvinyl chloride and Saran imparted an undesirable taste. Teflon was undesirable because it could not be used to form a heat seal. Thus, out of the seven films investigated only Mylar polyester and Trithene were of possible use.

Akers and Hopping (1958) found that aluminum foil had resistance to melting at temperatures above 121.1°C as well as barrier properties to oxygen and moisture that suggested its possible use in film laminations.

Keller (1959) when working for the U.S. Army Natick Laboratories, after testing single films and laminations concluded that there were more unsatisfactory films for heat-processed foods than those which showed promise. This was primarily because some of the films exhibit poor mechanical properties in the finished package because of their crystalline nature or orientation; examples of some of the unsuitable films were single films of high-density polyethylene, polyester and perfluorocarbon resin. Low-density polyethylene alone or as a laminate could not withstand temperatures greater than 115.6°C (240°F) and degradation of the film and breakdown of the laminate occurred in the presence of fat-containing foods. All single films tested were

deficient in barrier properties (that is, permeability to oxygen and moisture) in comparison to laminates, especially those containing foil. Food packaged in these films developed a poor color within 3 weeks in comparison to 6 months or more for laminates. Thus, the foil laminated package was found to be useful in preventing the development of oxidative flavor changes and moisture loss.

Gould et al. (1962) studied 6 films for packaging of snap beans and cream style corn. Of the 6 laminations tested: polyester/intermediate density polyethylene, polyester/polypropylene, polyester/foil/vinyl, polyester/foil/vinyl-improved, polyester/foil/polypropylene, and polyester/polypropylene, only the last two--polyester/foil/polypropylene and polyester/polypropylene were considered best for retort processing because of their greater ability to withstand retorting, greater durability during storage and higher quality of the products enclosed.

Felmingham (1964) studied Nylon 11 and polypropylene and found them undesirable because of the development of off flavors caused by oxygen permeability.

Long (1962) after reviewing the different classes of plastics available and their properties, concluded that it was impossible to attain a shelf life greater than 6 months without incorporating aluminum foil into a laminated film structure. The U.S. Army Natick Laboratories confirmed this by selecting a 3 layer lamination containing 0.5 mil polyester/0.35 mil aluminum foil/3 mil polyolefin (heat sealable component) for military development of the flexible package for army rations.

Thorpe and Atherton (1972) also studied 5 laminations: polyester/cast polypropylene, Nylon/cast polypropylene, modified

Nylon/cast polypropylene (all transparent), polyester/aluminum foil/ cast polypropylene, and polyester/aluminum foil/propylene copolymer (both opaque). Of the transparent laminations modified Nylon/cast polypropylene gave the best storage life of approximately 3 months. For the foil pouches the polyester/foil/propylene copolymer was better and had a shelf life in excess of 1 year.

In the 3 layer lamination the inner ply is required to provide a hermetic seal of the inner surfaces, a surface not readily subject to degradation by the food product and a high melting point to prevent seal failure during retorting. The external ply must mechanically protect the foil from puncture, tearing, corrosion, flexural damage and must be temperature resistant, hydrophobic, stable and durable. In order to provide a shelf stable product comparable in shelf life to a can, a lamination containing aluminum foil must be used. However, with the two plastics, one on each side protecting the foil from damage a very thin layer of foil can be used, i.e. 0.35 mil = 0.00889 mm (0.00035 inches).

B. Thermal Processing

Davis, Karel and Proctor (1960) pointed out that heat processing food in flexible films presented problems not encountered with conventional containers. These were:

1. permeability of films to gases and vapors and the effect of processing temperatures on these properties;
2. possible extraction of film components into the food product during processing;
3. effect of transmitted light on the food product;

4. essential inertness of plastics - in some food products the reaction between the food and the tin is beneficial and desirable (an example is pineapple color which is obtained by the bleaching action of the tin);
5. effect of processing temperature on the strength of films and heat seals;
6. internal pressure-volume relations in film package during processing.

Nelson and Steinberg (1956) first mentioned problems associated with sealing of foods in flexible packages. The first problem was that of obtaining uniform seals that did not weaken the film next to the seal, so that internal pressures of 34.47 k Pa (5 psig) would result in leaks. The second major problem occurred during filling. If food material remained in the seam area a good seal was impossible. Long (1962) also describes problems that can be encountered in filling and sealing. The pouch must be maintained upright before sealing to prevent contamination of the seal area. For food products involving both solids and liquids these have to be added separately to get the best control of fill. Gould et al. (1962) also found that when the top seam was damaged by the sealer or there was product in the top seal area the percentage recovery of pouches was reduced (top seam damaged, 67-83% recovered; product in seam, 91-97% recovered). Nieboer (1970) mentioned that it was important to prevent creases forming in the seal area when the seal is being made.

Andres and Duxbury (1972) described the automatic line filler that the U.S. Army Natick Laboratories developed to eliminate

contamination in the seal area. Four fillers were developed for pumpable, placeable, extrudable and bakery type products. The pouches were filled from the bottom up by inserting a tube into the pouch from the top and then raising the tube as filling took place. There was positive cut off of the product by the presence of a vacuum line which removed any excess liquid from the tip of the tube and eliminated drippage onto the seal area and in the area of the filling station.

There are three important physical factors in retorting flexible films: the amount of heat, the amount of pressure maintained and the length of the process. All of these factors affect the pressure-volume relation in flexible pouches during heat processing. Nelson and Steinberg (1956) first mentioned the need for processing with superimposed air pressure to prevent bursting of pouches. Another reason for overpressure is to minimize expansion of gas and vapor bubbles in the product which would interfere with heat transfer. Davis, Karel and Proctor (1960) indicated that the pressure which develops inside the sealed containers during heat processing arose from:

1. increase in the vapor pressure of the water in the processed food with increasing temperature;
2. increase in the pressure of air in the headspace with increasing temperature;
3. release of additional air from the product, due to a decrease in gas solubility with increasing temperature;
4. thermal expansion of the food product itself.

Davis *et al.* found that differences in pressures inside and outside film containers filled with water and several food products during processing at temperatures above 100°C under steam pressure were relatively small,

except during the cooling operation. This was because the retort temperature and pressure fell more rapidly than the temperature and pressure inside the pouch. Cooling under superimposed air pressure was found to avoid the development of high pressures inside the package. Keller (1959) carried this point further by stating that,

> "If headspace air is not substantially removed from a package, a commercial pressure water cook with pressure cooling must be used."

This requires a superimposed air pressure to prevent expansion and possible bursting of the packages and to insure proper heat transfer. Long (1962) stated that removal of headspace gas from pouches prior to closing was necessary to avoid the formation of occluded gas released at elevated temperatures that might interfere with efficiency of the heating cycle, and to hinder ballooning of pouches as well as the tendency for them to float. Removal of headspace gas can be attained either by injection of steam into the pouch from a superheated steam line with immediate sealing or, by pulling a vacuum on the pouch and while the vacuum is maintained, sealing the pouch.

There were various reports in the literature concerning the proper amount of overriding air pressure to be maintained during processing. When processing at 121.1°C, 103.42 k Pa (15 psig) is supplied by the pressure of the steam and the suggested overpressure required to prevent bursting of the pouches and to insure proper heat transfer varied from 48.26 - 103.42 k Pa (7 - 15 psig) additional pressure. With the addition of 48.26 - 103.42 k Pa (7 - 15 psig) a total gauge pressure varying from 151.68 - 206.84 k Pa (22 - 30 psig) would be attained, or up to about 3 atmospheres absolute.

Precautions that should be taken in order to reduce or eliminate the damage caused to a pouch by high internal pressure during processing are:

1. superimposed air pressure so the total pressure is 172.37 - 206.84 k Pa (25 - 30 psig);
2. removal of headspace air by vacuum or steam injection;
3. the filling of viscous products into pouches at elevated temperatures to reduce the amount of entrapped air.

Long (1962) and Gould et al. (1962) were the authors to first mention the need for a rack on which to place the pouches, in the retort. Long stated that the rack should be designed to permit maximum heat-penetration of all package surfaces and allow room for pouch expansion or for change in shape in any direction and that the pouch surfaces should not contact each other or carry any weight of the rack. Pflug, Bock and Long (1963) went into greater detail on the necessity for a rack. As the pouches are buoyant the water currents will tend to pile them together and they will not all receive uniform heating. To keep process time to a minimum every pouch must be exposed uniformly and separately to the heating medium. The thickness of the filled pouch will govern process requirements as the point of greatest temperature lag will be at the center of the thickest part. To establish reproducible heat penetration curves, pouch dimensions must be controlled. This can be done by placing pouches in a rack that has a standard thickness. Andres and Duxbury (1972) mentioned that the carriers that the U.S. Army Natick Laboratories developed were designed to handle the pouches automatically from filling to the completion of retorting.

Pflug et al. (1963), Thorpe and Atherton (1972), Rees (1973) and Davis, Long and Robertson (1972) all discussed whether pouches should be retorted vertically or horizontally. Pflug and Davis were in favor of a vertical position of pouches to ensure adequate circulation of the heating medium. They pointed out that pouches in a horizontal position required a longer process because of the difficulty of forcing the water to circulate in a horizontal direction. However, Thorpe and Atherton, and Rees both (loc. cit.) stated that pouches containing brine or those of a fairly fluid nature would tend to 'bow' with subsequent increase in cross-sectional area at the base of the pack. In a bulged pack particulates would tend to collect together at the base of the pack which in turn would hinder convection currents set up in the pack and so the contents of pouches processed in a vertical position would take longer than those processed in a horizontal position to achieve the same degree of processing. Rees concluded that for the majority of products, optimum processing conditions were attained when pouches were processed horizontally when minimal cross-sectional dimensions and an even rate of heat transfer are achieved. Thorpe and Atherton stated that root crops packed without any covering liquor and vacuum sealed, which retain their original overall shape, may be processed either horizontally or vertically without affecting the rate of heat transfer.

The choice of heat processing media is between processing in water with a superimposed air pressure, or in a steam-air mixture supplying its own overpressure. This is different from a regular steam cook which has no air pressure. Pflug et al. (1963), Turtle and

Alderson (1971), Davis et al. (1972) and Rees (1973) discuss the merits of the two methods. The advantages of the steam-air cook are:

1. quicker and cheaper, and
2. flexible packages do not float.

The disadvantages are:

1. a homogeneous steam/air mixture must be attained to prevent underprocessing, and
2. this homogeneous steam-air ratio must be reproducible every time a retort load is processed.

However, Pflug et al. (1963) used the steam-air cook successfully and Tsutsumi (1972) and Rees (1973) indicated that the Japanese seem to be successful in using a steam-air cook and have a thermal distribution throughout the retort of ± 0.5°C to ± 2.0°C.

The disadvantages of the water cook are:

1. the process is longer due to the extra time to heat and cool the water;
2. water hardness may soil pouches and build scale on racks;
3. improper addition of steam to the water can cause flexure fatigue damage to the pouches; and
4. circulation of water is required to prevent layering either by using a pump or by introducing air through steam spreaders at the bottom of the retort.

The advantages of the water cook are:

1. simple and foolproof, and
2. uncomplicated.

Turtle and Alderson (1971) and Davis et al. (1972) both recommended its use.

In order to estimate the process necessary to achieve microbiological stability in flexible food packs that require pressure processing, a knowledge of the history of time vs temperature at the center of the pack is required. Heat penetration studies must be done for each product, pouch size, rack dimension and retort hookup. To obtain information concerning the heating rate a thermocouple, usually copper-constantan, is placed at the slowest heating point in the pouch and the time-temperature relationship is determined with a potentiometer during the heating and cooling cycle. Thermocouples can be introduced into the pouch by hermetically sealing them with an adhesive either into the side or bottom pouch seal as described by Pflug et al. (1963). The thermocouple was maintained in a central position by support from a gusset. The disadvantage of introducing a thermocouple through the seal is that seals often failed during processing as a result of pouch movement. To overcome this problem, Pflug et al. (1963), Turtle and Alderson (1971), and Davis et al. (1972) described the use of a metal packing gland or nylon packing gland which is more expensive, but which can be reused many times and which is quick and simple to install. The thermocouple wire is fed through the gland and tightened after a sufficient length is inside the pouch. The thermocouple is maintained in position by aid of a plastic saddle or an M shaped gusset made of autoclave tape, or by locating the thermocouple tip in the center of a coiled spring for liquid or semi-liquid food products. For particulate or solid foods the thermocouple is simply inserted between slices or in a piece of food product. Thorpe and Atherton (1972) sealed the introduced thermocouples through a small hole near the bottom seal with

adhesive. The thermocouple tip was held in position by a gusset for solid food or by Nylon spacers for particulate food.

From the heat penetration data, the uniformity and adequacy of heat penetration can be determined. Microbiological stability for low acid foods (pH greater than or equal to 4.5) is defined as that condition in which all Clostridium botulinum spores and all other pathogenic bacteria have been destroyed as well as more heat resistant organisms which, if present, could produce spoilage under normal conditions of storage and distribution. In the determination of a heat process for pouched food two methods may be used, firstly the experimental pack procedure, and secondly calculations which are based on the heat penetration and the thermal death time data. The experimental pack procedure involves the inoculation of the pouched food with bacteria of known heat resistance, processing at different levels of time and/or temperature and determining the degree of spoilage by incubation or subculturing. The calculation method involves the correlation of heat penetration data which can be achieved by the method developed in 1928 by Ball (quoted by National Canners Assoc. Research Laboratories, 1968). In this method straight line logarithmic curves of the thermal death times are used. Four symbols describing these curves given by the National Canners Assoc. (1968) are:

1. D = the time required at any temperature to destroy 90% of the spores or vegetative cells of a given organism, or the number of minutes for the survivor curve to traverse one log cycle.
2. F = the number of minutes required to destroy a given number of organisms at a given temperature.

3. F_o = the number of minutes required to destroy a specified number of spores at 121.1°C (250°F) when z = 10°C (18°F).
4. z = the reciprocal of the slope of the thermal death time curve expressed as °C, or the temperature interval required for the line to pass through one log cycle.

The temperature from the heat penetration data are measured every minute and these are converted into lethal rates. These lethal rates summed, give the total lethality in terms of F values. With anaerobic spore-formers with a z value of 10°C (18°F) like Cl. botulinum a minimum treatment equivalent to 121.1°C (250°F) for 3 minutes or an F_o = 3 is considered necessary to produce a microbiologically stable product. With root vegetables or low acid vegetables in contact with the soil Cl. botulinum is the pathogen most likely to be associated, so an F_o = 3 should be attained (Thorpe and Atherton, 1972).

Tsutsumi (1972) described in detail the development and use of flexible pouches in Japan. An automated batch retort system using a steam cook with overriding air pressure was used to sterilize the pouches, which were placed on horizontal aluminum plates pierced with holes. These plates were stackable to form a rack. Each batch could process about 2000 pouches. The temperature was controlled within ± 2°C. Filling was conducted by a filler-sealer which had six stations consisting of picking up a pouch, opening, filling, presealing, post-sealing and cooling with an operation rate of 30 - 35 pouches/minute. The air was reduced in the pouches by stretching both sides of a pouch just before sealing. The pouches were manually checked for a good seal to ensure that bubbles, wrinkles and food particles were not present

before and after heat treatment. Next the filled pouches were individually packed in outer cartons to protect the contents from breakage during shipping, stacking, and display.

Guedez and Bates (1975) described a simplified procedure for processing flexible pouches that can be performed on a pilot scale without specialized equipment or controls. A vertical retort using a steam cook with a stainless-steel rack was used. An overriding pressure of 6.89 - 13.79 k Pa (1 - 2 psig) was used above the processing pressure, 103.42 or 172.37 k Pa (15 or 25 psig). Copper-constantan thermocouples were used to obtain heat penetration data and were inserted in the geometric center of the pouches through a packing gland. In order to introduce the overriding air pressure the retort was modified by adding an air pressure valve and a quick opening valve. The air pressure served to purge the retort of steam prior to admitting cooling water and prevented pressure drop due to condensation and also provided an overpressure of 6.89 - 13.79 k Pa (1 - 2 psig) above the processing pressure. Guedez and Bates stated that the main advantage of this method over the steam-air cook is that control of pressure and of media circulation and mixing is unnecessary. However, they implied that the low volume of pouches produced or tested (c. 300) did not ensure the method was foolproof in its present form. Pouches made of polyester/aluminum/Nylon and polyester/aluminum/polyolefin both performed well. Guedez and Bates concluded that the use of flexible pouches could be readily used for low through-put and laboratory process applications or in developing countries, where difficulty might be experienced in obtaining various sizes of cans and jars and also that

the pouches would take less space and weight. They also recommend the technique for conducting exploratory studies and preparing samples before investing in sophisticated prototype or industrial systems.

C. Package Integrity, Storage Trials and Quality Assessment

Many tests have been developed in order to provide assurance that the thermo-processed shelf stable foods in flexible pouches will have a shelf-life comparable to cans and will withstand shipping and transportation handling.

Keller (1959), Turtle and Alderson (1971) and Thorpe and Atherton (1972) stated that the use of an outer package, container or folder is necessary for the flexible pack to withstand impact abuse during manufacturing distribution and retailing, thereby preventing snagging and puncturing of pouches and to prevent microbial contamination of external pouch surfaces after heat processing.

The major reason for flexible package failure would be defective seals caused by formation of wrinkles or by contamination of the seal by food product. Contamination by food can be reduced or eliminated by using specially developed product fillers matched to the food product characteristics, having bottom to top filling, controlling the configuration of the pouch opening when filling, being careful to handle pouches so as to reduce splashing, and being careful in removal of air so that splashing is reduced. Lampi et al. (1976) emphasized the need today, for greater attention to the formation of seals and measures of their performance as a result of requiring leaker rates as low for pouches as for metal cans, seals that must withstand 120°C or higher thermoprocessing temperatures, packages that must be durable through

the entire distribution system and extended shelf life requirements. These authors described four main criteria or testing techniques for a good flexible pouch seal which are fusion testing, burst testing, tensile testing and visual examination.

Fusion exists when the opposing seal surfaces form a total weld. Examination is done visually before and after applying tension beyond the point of failure. The internal burst test involves filling the pouch with air to a predetermined level using either the pressure to burst, time to burst at a constant pressure or the withstanding of a preset pressure time cycle. The tensile test can best be used for surveillance of the sealability of materials and as a spot check on sealing conditions and equipment operation. It measures the total force or weight required to cause failure over the total width of the sample strip. Lampi et al. (1976) recommended using the tensile test supplemented by burst tests. There are no subjective or objective nondestructive methods for assessing seals for fusion, tensile or burst strength, so reliance must be placed on periodic destructive testing.

An automatic inspection system has been developed by the U.S. Army Natick Laboratories to detect seal defects and is described by Lampi et al. (1969, 1973, 1976). The system involves infrared radiometric scanning of transiently heated seal surfaces to detect a variety of defects such as small occluded particles, wrinkles, voids, non-continuous fusion across the seal width, and misaligned packs. Although infrared scanning is the most accurate method to detect seal defects the cost is high especially when rejecting defective seal units. Two other methods of detecting physical aberrations and contaminations in

seal areas are visual examination and caliper scanning. The visual method is the least expensive but has seal defect detection rates of 0.011% - 0.02% with 100% inspection of pouches which is better than the accepted failure rate of 0.1% for cans. Caliper scanning uses calipers to measure irregularities in seal thickness, and although less costly than infrared, the particles and wrinkles detected must be larger than for infrared, and alignment must be accurately and closely controlled. Based primarily on failure rates noted after retorting and during rough handling, Lampi concluded that the minimum seal width should be 0.64 cm and that occluded particles cannot be tolerated when testing 0.32 cm and 0.64 cm seal widths.

A second area requiring testing involves package abuse, which refers not only to failure of seals but more particularly to failures of the pouch body due to flexural fatigue, impact shock, abrasion, snagging or pinholing during manufacturing or at any later stage in the life of the pouch. During manufacturing, package abuse can be reduced by having a satisfactory layout and conveying system, and by having all equipment that contacts the pouches smoothly finished. Tests that have been developed to determine the resistance of the flexible package to abuse include the drop test, tumbling tests, abuse retorting tests, vibrator, rotator, revolving hexagonal drum and guided drop tests. Turtle and Alderson (1971) found that there was no failure at drop tests of 243.8 cm (8 ft) or tumbling tests at 12 rpm for a flexible package containing 120 g of meat which was glued in a protective 0.406 cm (0.016 in) caliper board carton. Szczeblowski (1971) reported that: for abuse retorting which is the fluctuation of pressure by

± 13.79 k Pa (2 psig) every 2 minutes for 30 minutes, 0/50 leaked. For the vibrator test which vibrates at 268 cycles/minute, 6 pouches were glued to a board and vibrated for 1 hour, no pinholes were produced. For the rotator test 4 pouches containing liquid were glued to a board and rotated at 30 rpm. As they were rotated they were flexed at the same point each time. After 39,400 revolutions, the packages were discolored but showed no leaks. With the revolving hexagonal drum test the drop height was 50.8 cm (20 in) and damage was by abrasion as the packages were flipped from one side to the other. For retorted pouches in fibreboard folders, after 7,920 drops or 11 hours the corners of the folders were wearing away. The guided drop test involves repeated drops on edges, faces and corners of pouches without folders. Although pouches showed flexing, and foil breaks were observed, no complete breakthrough was found and no bacterial penetration was noted after biotesting (see below).

Burke and Schulz (1972) made a direct comparison of metal cans and flexible packages in a laboratory rough handling test involving the vibrator and drop tests. The test showed equal performance of metal cans and flexible pouches based on bacterial penetration after biotesting. Superior performance was obtained with flexible pouches when dented cans were considered defective.

Biotest procedures as described by Thorpe and Atherton (1972), Szczeblowski (1971), and Maunder, Folinazzo and Killoran (1968) to detect any leak through which micro-organisms could enter and cause spoilage of the pouch contents, involved immersing the filled and processed pouches in a liquid containing a high concentration of a gas

forming micro-organism and subjecting them to a flexing action to allow bacteria located at a point of microleakage to be drawn into the package.

Shipping tests were described by Goldfarb (1971), Szczeblowski (1971) and Thorpe and Atherton (1972) and it can be concluded from these tests that flexible packages can be considered satisfactory in regard to shipping and handling by all means of transportation.

The U.S. Army Natick Laboratories possess samples of food processed in flexible pouches that have been stored for 8 years and which have shown no visible sign of deterioration. Thorpe and Atherton (1972) also did storage trials and found shelf lives of 18 months easily attainable in foil laminated pouches.

Plastic materials of high heat resistance offer unique advantages in the food packaging field. These are:

1. weight and volume reduction in transport and storage of both empty and filled containers;
2. improved quality, flavor, color, nutritive value and texture of the product as a result of minimal amount of liquid in the pouch, so that leaching of nutrients is decreased and shorter processing times can be used;
3. convenience to campers and other consumers by boil-in-bag feature that allows several products to be heated in 1 vessel of water with boiling for only 3 - 5 minutes;
4. easy opening with aid of tear notch or scissors;
5. simpler disposal of pouches than of cans and jars, and less requirement of storage space;
6. ease in changing container capacity and in providing portion control for the consumer;

7. better marketing presentation on the increased surface area of the outer protective carton;
8. no requirement of special storage temperature for complete recipe packs that are at present available only in frozen form.

As there are advantages so must there be disadvantages; these are:

1. flexible packages require more sophisticated heat processing equipment;
2. more careful handling is needed and the flexible packs require protection by an external carton or folder;
3. extensive trial marketing may be required to get the public to accept flexible packages as readily as they accept cans and jars;
4. there is a lack of high speed form fill and seal machinery in comparison to speeds attainable by cans and jars, 35 pouches/minute vs 1200/minute for jars and 200 - 400/minute for cans (Mermelstein, 1976);
5. automatic handling systems that are available have high capital costs;
6. fragile products may not be protected sufficiently during distribution;
7. flexible pouches would not be suitable for large packages of fluid or semi-fluid products;
8. the cost of a flexible pouch is estimated to be 20% more than the cost of a comparable can, however the price of tin is increasing faster than the cost of plastic so pouches may soon

become comparable or even cheaper. Knight (1971) compared the cost of a can, label and processing at 13.8¢ against a pouch with over-carton, and processing at 13.4¢, but no account was taken of shipping and inventory costs. Mermelstein (1976) stated that the cost of the flexible pouch is more expensive from a volume standpoint and because of the required overwrap, but since the cost of materials for cans is increasing more rapidly than for a pouch the cost difference will lessen. Further economies in total pouch cost will result from higher line speeds, eventual elimination of the overwrap, and the opportunity to market the product in multipacks.

The retort pouch is just about to be marketed in the U.S. The marketing plans have been held up by a last minute review concerning the migration of components of the adhesive through the food contact ply of the 3 ply lamination and because of information that was made available to them by one of the suppliers of pouches that migration of extractables would take place at retort temperatures, 115.4 - 132.1°C (240 - 270°F). Thus, until the FDA amends the existing regulations covering the various materials that can be used in pouches, interstate marketing of flexible pouches in the U.S. will not occur.

III. MATERIALS AND METHODS

A. Potatoes

Tubers were obtained from three sources: a local potato processor; the Alberta Potato Commission; and a local supermarket. Potatoes were stored in a humidity and temperature controlled chamber until needed (90% and 4.4°C respectively). Prepeeled and bisulphite treated potatoes from the processor were used within 24 hours.

B. Flexible Packages

Two types of flexible pouches were used throughout the study: Sterilite NP and Sterilite NFP, both obtained from E. S. and A. Robinson (Canada) Ltd. (Toronto). Both types were 24 x 16.5 cm size. The pouches consist of 2 or 3 layers held together by an adhesive, such as polyurethane (see Fig. 1). Sterilite NP is a transparent lacquer lamination of Nylon and cast polypropylene. The Nylon film gives toughness, clarity and low permeability to oxygen and the cast polypropylene is the heat seal component. The Sterilite NP was primarily used for its transparent property. Sterilite NFP is a lacquer lamination of Nylon film to aluminum foil which is laminated on the reverse side of the foil to cast polypropylene as the heat sealable component. Sterilite NFP is designed for hard vacuum packed foods with a longer shelf life than Sterilite NP.

C. Cultures

The test organisms were anaerobes, *Clostridium sporogenes* (P.A. 3679) strains from Canada Packers and the Department of National Health

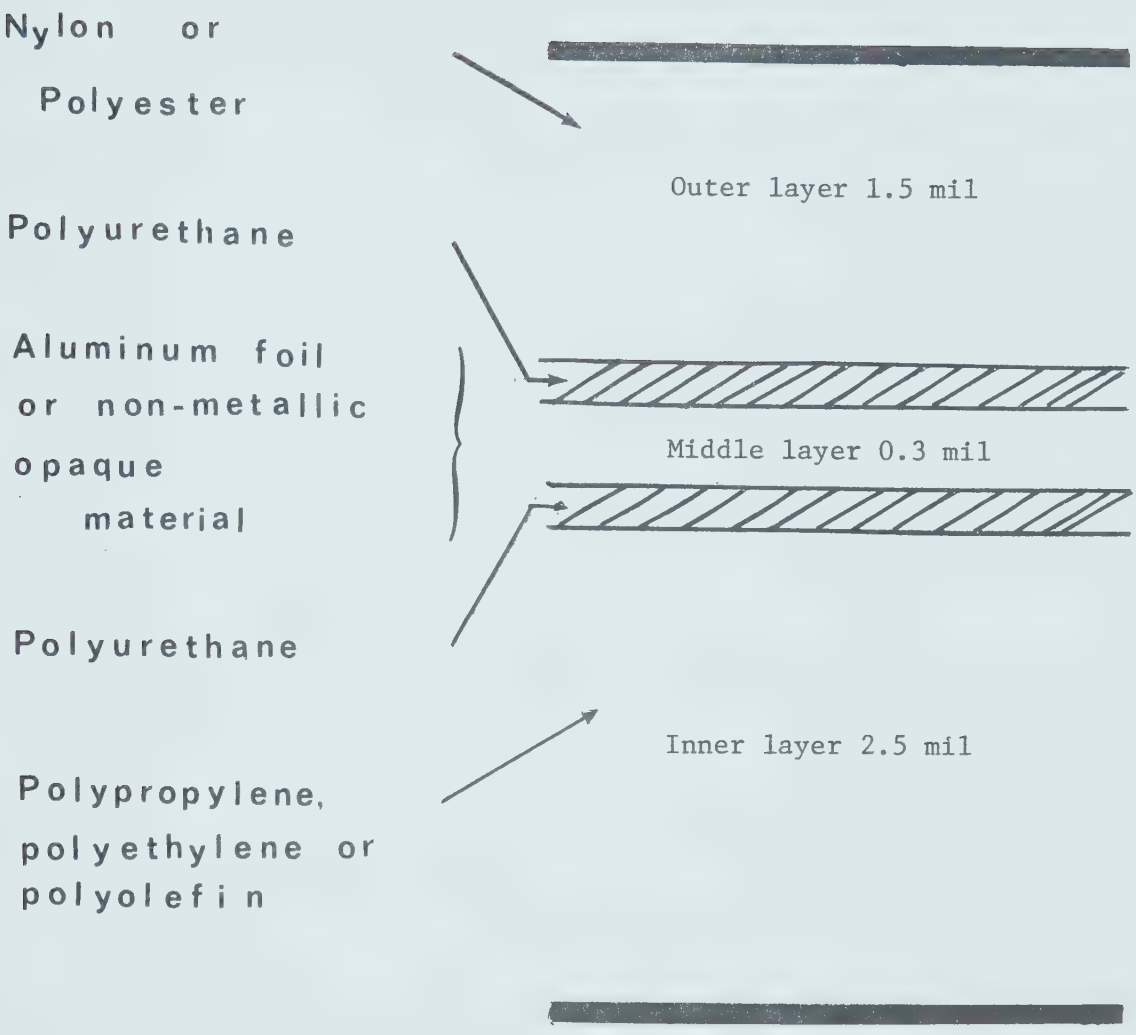

Fig.1. Cross-section of typical laminated film

and Welfare, Ottawa; Clostridium thermosaccharolyticum (7956) from the American Type Culture Collection, Rockville, Maryland, U.S.A.; and the facultative anaerobe Bacillus stearothermophilus (B7/202) obtained from the National Institute of Research in Dairying, University of Reading, England and strain 8157 from the National Collection of Industrial Bacteria, Scotland. The above strains were grown and tested until a suitable quantity of heat resistant spores was obtained.

D. Media

Four types of media were examined to produce crops of spores.

1. Oxoid Reinforced Clostridial Medium (RCM) (BBL, Codkeysville, Maryland, U.S.A.) was used to produce spores of P.A. 3679 and also was used to determine the naturally occurring anaerobic counts on potatoes.
2. Trypticase Broth (King and Gould, 1971) was used as an alternative to produce spores of P.A. 3679.
3. Pea Broth (Folinazzo and Troy, 1954) was used to cultivate Cl. thermosaccharolyticum.
4. Supplemented Nutrient Agar (Finley and Fields, 1962) which was used to produce spore crops of B. stearothermophilus contained nutrient agar 1.5%, supplemental plain agar 0.5%, glucose 0.05%, (all w/v) and $MnSO_4 \cdot H_2O$ 30 ppm.

E. Propagation

1. P.A. 3679

The cultures were grown in 100 ml quantities of sterile RCM and incubated at 37°C. They were examined daily for production of spores by the Gram stain. When spore production proved to be poor the trypticase

broth (King and Gould, 1971) was tried. The test tubes containing 25 ml of media were inoculated and checked daily for spore production. After six days a dense crop of spores (greater than 1 x 10^6/ml was obtained).

2. Cl. thermosaccharolyticum

Difficulty was experienced in obtaining a satisfactory crop of spores using sterile pea broth, but before the difficulty was solved a satisfactory crop of B. stearothermophilus spores was obtained (see below).

3. B. stearothermophilus

Freeze-dried cultures of B. stearothermophilus (8157) failed to produce a satisfactory spore crop in flasks of nutrient broth. However, a preparation of B. stearothermophilus (B7/202) received from the National Institute for Research in Dairying, Reading, grew successfully in Roux flasks containing 200 ml of supplemented nutrient agar and within a week produced a profuse growth of spores.

F. Harvesting

P.A. 3679 was harvested by pouring the spore media suspension into 200 ml centrifuge bottles and harvesting by centrifugation in a refrigerated centrifuge (Sorvall RC-2B) at 10,000 rpm for 10 minutes. The supernatant was decanted and the cells resuspended in sterile distilled water and washed three times with sterile distilled water before storing them at 5°C.

B. stearothermophilus spores were harvested from the surface of medium in Roux flasks using sterile glass 'hockey sticks' and sterile distilled water and placed in sterile centrifuge bottles. The initial speed used in the refrigerated centrifuge was 10,000 rpm but due to the lack of formation of a hard pellet higher speeds were tried until 16,000 rpm for 10 minutes was found to be satisfactory. The spores were centri-

fuged and washed several times with sterile distilled water before storing suspended in glass distilled water at 5°C.

G. Heat Treatment

The method proposed by Bigelow and Esty (1920), quoted by Stumbo (1965), with modifications similar to those described by Prentice and Clegg (1974) was followed. One and a half ml amounts of the working suspension were introduced into 2 ml sterile thin glass ampoules (Wheaton Glass Company, Millville, New Jersey, U.S.A.) by means of long tip graduated 5.0 ml pipets. After sealing the ampoules with a propane torch, they were completely immersed in a constant temperature oil bath containing paraffin, at the selected temperature for selected times. On removal they were cooled immediately by placing in ice water.

H. Colony Counts

Serial dilutions of the heat-treated spores were made in 9 ml amounts of 0.1% (w/v) sterile peptone water and 1 ml of suitable dilutions were plated using Standard Pour Plate technique in triplicate with supplemented nutrient agar. Initial incubation was done at 55°C until the optimum temperature for heated B. stearothermophilus spores was determined to be 47°C (see section IV, D). The maximum number of colonies formed was counted at 48 hours and daily until 72 hours, after which no increase in colony count was observed.

I. Inoculation of Pouches

Pouches of peeled potatoes after sizing, and weighing were inoculated with c. 5×10^7 B. stearothermophilus spores per pouch. This

was done by dripping 1 ml of the pure spore suspension over the potatoes and palpating. The pouches were then vacuum sealed.

J. The Retort

Preliminary work dealing with the feasibility of using a small fully automatic vertical retort to process potatoes in flexible pouches was done with superimposed air pressure, 6.895 k Pa (10 psig), using a steam cook. This was later modified to a water cook with superimposed air pressure because of the unacceptable temperature variations found within the retort without any mixing of the steam atmosphere. The modification involved addition of a water circulation pump, hot water source, and water level indicator. The retort, although modified, could still be used as a steam cook with or without air overpressure. The design of modifications to the retort was drawn up by Dr. L.F.L. Clegg, based on recommendations from the Continental Can Company Inc. (1969). The modifications were made with the guidance of Mr. Ron Phillips then Director of Physical Plant at the University of Alberta. Tests for the suitability of these modifications and the temperature variation in the retort were made by the present author.

In order to process pouches of potatoes the following steps were taken after a rack of pouches was placed in the retort (see Fig. 2):

1. close two inside drain valves (1) and two outside exhaust valves (2);
2. turn on hot water (3);
3. fill to within 8 in of the top and turn off the water and preheat to 90°C;
4. close lid and open petcock (4);

1. Drain Valves
2. Exhaust Valves
3. Hot Water
4. Pet Cock
5. Pump
6. Air Valve
7. Air Condenser
8. Automatic Steam Valve
9. Controller Timer
10. Power Switch
11. Start Button
12. Red Light
13. Air Valve to Retort for Over Pressure
14. Cold Water
15. Water Level Indicator
16. Constant Level Device
17. Thermocouple Exit
18. Pressure Gauge
19. Pressure Release Valve
20. Exhaust Timer
21. Control Element
22. Thermometer
23. Steam Spreader
24. Pressure Regulators

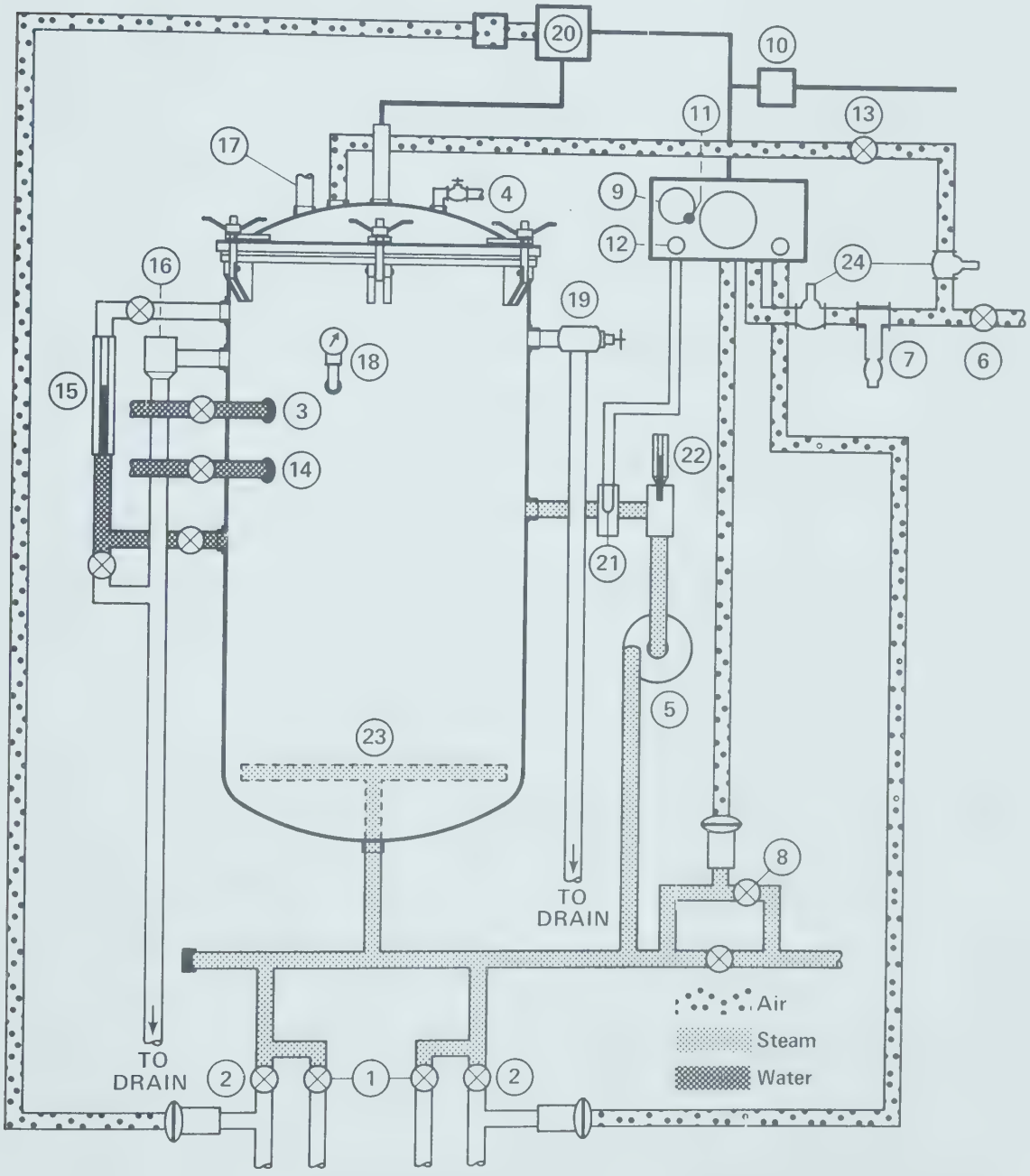

Fig. 2 The automatic steam retort as modified for the water cook process with air over-pressure

5. turn on pump (5);
6. turn on air valve (6), open and close condensate trap (7);
7. turn on automatic steam valve (8);
8. set controller (9), switch on electricity (10), and press red button to start (11);
9. close petcock;
10. wait until the temperature is 121.1°C (250°F), red light comes on (12);
11. turn on air which is maintained at 172.37 k Pa (25 lb) by the regulator (13);
12. after 20 minutes, or the desired time, the controller cuts off;
13. partially open cold water valve (14);
14. partially open a drain valve (1) and watch water level (15);
15. fully open cold water valve;
16. open drain valves more, again watching water level;
17. when the temperature is well below 77°C (170°F) turn off cold water and close drain valves;
18. turn off air pressure;
19. wait until the pressure drops to 13.79 k Pa (2 lb), then open petcock;
20. stop pump;
21. open drain valves fully;
22. open lid;
23. switch off electricity, turn off automatic steam valve and air valve.

The retort was an important tool in this research because it was used where possible as a 'laboratory' instrument for heat resistance tests for pouches and for micro-organisms on the surface of potatoes in evacuated pouches.

IV. RESULTS AND DISCUSSION

A. Heat Penetration Studies

1. Uniformity of heating in the retort

Uniformity of heating throughout the retort was determined by using twelve copper-constantan thermocouples hooked to a twelve level rack by paper clamps in a random arrangement. The rack was made of expanded metal mesh and thus allowed good circulation of the water. An illustration of the rack is given in Fig. 3.

Preliminary temperature determinations with thermocouples were done using twelve individual connectors. This was changed to one connector for the twelve connectors to allow the connector to be above the surface of the water; this reduced corrosion and the occurrence of short circuits. However, difficulty was experienced because this connector acted as a heat sink, so that thermocouple temperatures registered by the recorder were incorrect and could be changed radically by placing ice on the thermocouple exit. Thus, the next step was to return to the individual connectors except that the twelve connectors were inserted above the level of the water and placed in a heat resistent flexible pouch to reduce water condensation and short circuiting and to reduce movement when the cooling cycle began.

When these difficulties were overcome the temperature of the thermocouples vs time was registered by a Honeywell Electronik 112 Multipoint recorder. All the temperatures for processing were determined in °C even though traditionally processing information is given

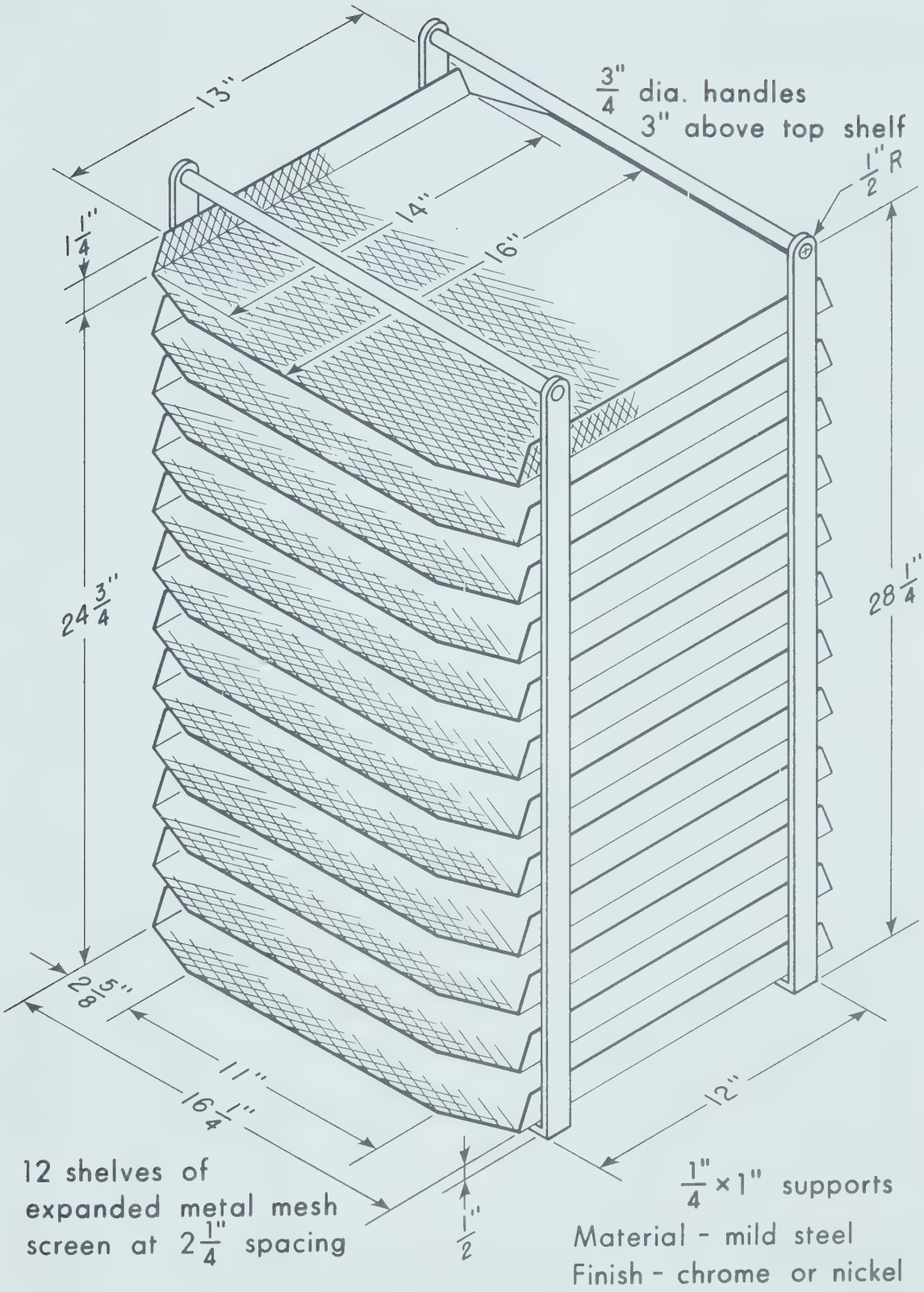

Fig.3 Retort rack used to support flexible pouches

in °F, because the recorder registered °C only. Once processing temperature (121.1°C) was reached the maximum range in fluctuation between thermocouples was found to be ± 1°C (2°F) (see Fig. 4).

As the heat processing times which were necessary to obtain sterilization depend to a large extent on the rate of heat penetration into a container, Wornick, Karel and Proctor (1960) obtained information on the rates at which heat is conducted into containers made of flexible plastic films. They concluded that the thermal resistance of the plastics tested (Mylar polyester, hi-density polyethylene and polypropylene), at least in the thicknesses used (10 - 30 mils), represented a negligible portion of the total thermal resistance and that heating time will be more nearly a function of the conductivity of the food and the shape of the container, with the plastic film offering little resistance. Chapman and McKernan (1963) compared heat conduction between high-density polyethylene trays of various wall thicknesses and cans of the same capacity (12.06 cm x 9.52 cm x 3.81 cm containers). They observed that there was little difference between plastic and tinplate containers of equivalent dimensions, provided the plastic container walls were less than 20 - 25 mils thick. Because the plastic laminated pouches used in this work differed from the materials tested by Wornick et al. and to determine if the pouches themselves hindered heat conduction, a comparison was made between six thermocouples attached to the rack after sealing them in empty pouches and six thermocouples which were attached to the rack without being in a pouch.

From the previous information and Fig. 4 there can be seen to be a negligible difference between the thermocouples that were not in pouches. Thus, the flexible pouches used did not hinder to any extent

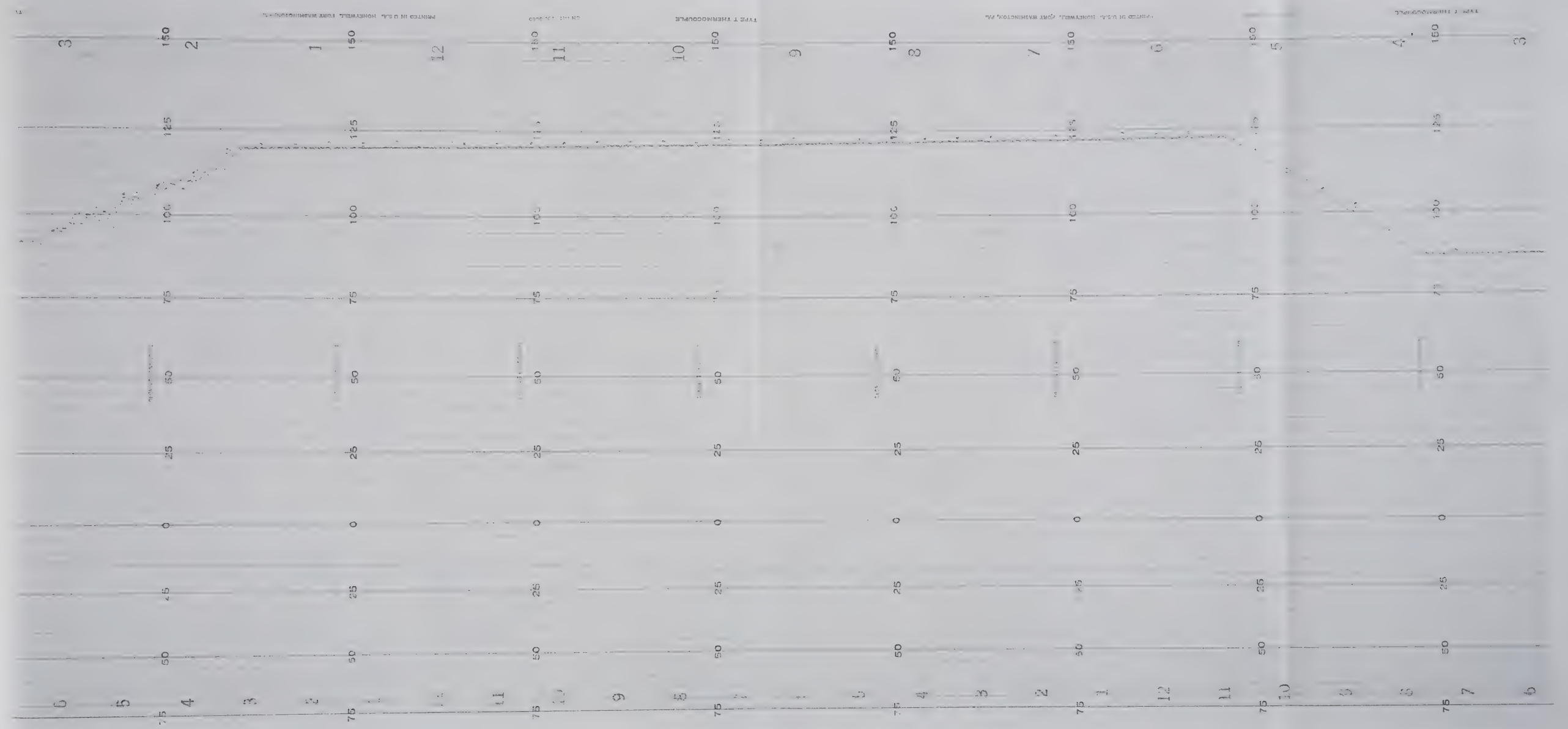

Fig. 4 Chart showing temperatures recorded by 12 thermocouples (6 sealed in pouches and 6 in water not in pouches) throughout the "coming-up", heating and cooling periods

the conduction of heat into the interior of the pouches as expected because they were less than the thicknesses tested above by Wornick et al. (c. 4 mils).

The uniformity of heating in the retort was determined in order to ensure that the pump used to force circulate the water was of correct size to prevent formation of horizontal isothermal layers of water of differing temperature. The maximum fluctuation between thermocouples randomly arranged throughout the height of the retort was ± 1°C, which was similar to the recommendation by Thorpe (1974) of not more than -0.55°C (-1°F) and +1°C (2°F) of the retort temperature, within 1 minute of the retort thermometer reaching process temperature.

Although trouble was experienced with the thermocouple connectors shorting out and going off scale on the recording there was not any method readily available to overcome this problem. The way around this was to use a sufficient number of thermocouples that even if a few were lost, there would still be enough thermocouple recordings to give reliable and reproducible results.

2. Uniformity of heat penetration between pouches of potatoes

To prepare flexible pouches to accept thermocouples, a laminate of transparent Nylon/polypropylene was used. The transparent pouches were used because of their ease of manipulation. A seal 3 in in length was made one inch from the right edge and one inch from the bottom of the pouch, using a CRC Rotary Heat Sealer (Chemical Rubber Co., Cleveland, Ohio). Then a seal at the top of this 3 in seal was made over to the right hand edge of the pouch. Approximately a 1/2 in square slit was cut out of the top layer of plastic between the 3 in seal and the edge of the pouch about 2 to 2-1/2 in from the bottom. The thermocouple wire was pushed through the slit, down the remaining length of the narrow strip and up into the larger portion of the pouch. The slit was sealed with epoxy glue and allowed to harden overnight (see Fig. 5). The thermocouple wire was held in place to prevent flexing with autoclave tape 2 to 3 in above the glue area.

Potatoes of the same specific gravity were used, less than 1.070. This was partly because this was the desired quality of potato to be used for this process so as to eliminate problems of poor flavor and sloughing evidenced by potatoes of specific gravities greater than 1.100, and partly in order to minimize any possible variation in heat penetration caused by differences in density of potatoes. The potatoes were peeled and trimmed so that they would pass through a 1-3/4 in diameter hole. The depth of the potato, which is the minimum dimension, was noted as the depth of the tuber is the controlling factor for thickness for a pouch of potatoes, and in turn controls rate of heat penetration.

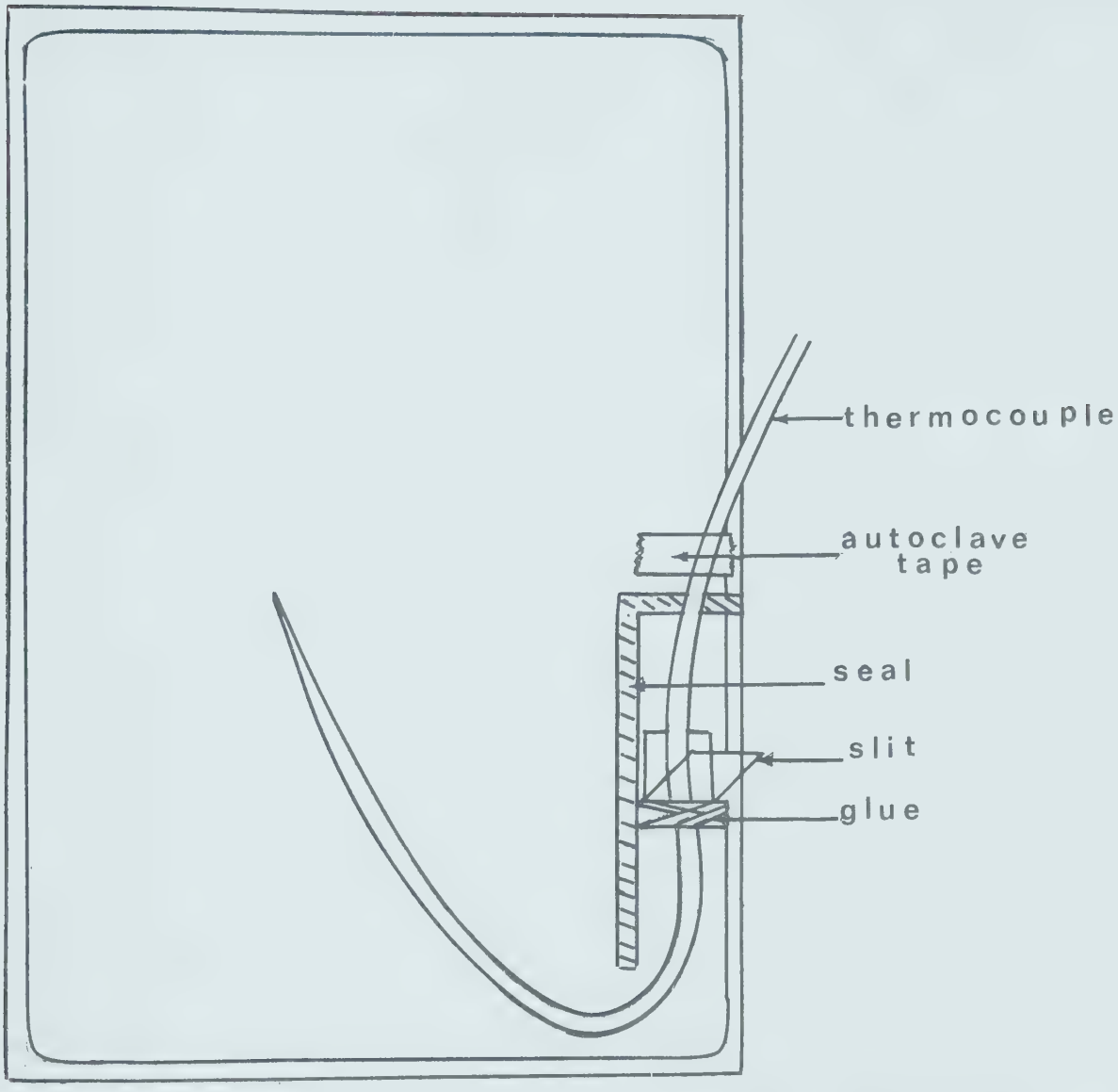

Fig.5 Method of sealing a thermocouple within a pouch

All the potatoes in a run were trimmed to the same minimum depth and width. The potatoes were then weighed as a safety measure in case a large difference in total lethality (F_o) could be related or explained by a difference in weight (Thorpe, 1972). A metal probe was pushed into the center of the potato by marking the probe at one half the depth of the potato by placing a thumbnail against the probe. This left a narrow bore to the center. Again, after marking one half the minimum depth of the potato by placing the thumbnail against the thermocouple wire the thermocouple wire was fed into this bore. One potato was put in each pouch carefully so as to not disturb the thermocouple.

Prior to sealing the top 2 to 3 in of the inside of the pouch was wiped with a tissue to remove any moisture or particles that would hinder formation of a good seal. The pouches were vacuum sealed using a Multivac M311 Vacuum Sealer from Knud Simonsen Industries Ltd., Canada and visually inspected for defects in the seal area which would have prevented the attainment of a hard vacuum. The pouches were placed horizontally on the rack and held in place with paper clamps. A minimum of 6 pouches was placed in the retort for each run, so that there would be at least one or two replicates if some of the thermocouples were lost (because of movement of water, etc.) causing the recording to go off scale or by the thermocouple tip moving from the center position. The position of the thermocouple was checked after each run for each potato by carefully cutting the pouch open and cutting the potato in half to determine if the thermocouple tip was at the center of the potato relative to depth or had become dislodged during processing. If the thermocouple was not in place the data were discarded. The pouches for each run were processed at 121.1°C for twelve or twenty minutes, and the temperatures

at the center of each potato was recorded every minute. As the thermocouple recordings were in °C it was necessary to calculate a table of lethal rates by using the formula

$$\text{lethal rate} = \frac{1}{\log^{-1}\frac{(250 - CT)}{z}}$$ (National Canners Association Research Laboratories, 1968) suitably modified to °C, so that it became

$$\text{lethal rate} = \frac{1}{\log^{-1}\frac{(121.1 - CT)}{z}}$$ where

CT = the temperature of the cold spot in the pouch in °C, that is the center of the potato

z = the number of °C for the thermal destruction curve to traverse one log cycle.

The temperature vs time recordings at the center of the potato were used to calculate the lethality of the process by converting temperatures at minute intervals to lethal rates and adding these to give the process values in F_o units by using the approximate formula, which is based on an approximation of the trapeze formula

$$F = \int_0^{\theta} L d\theta,$$

$$F = \frac{\theta}{2}\left(L_o + \sum_{i=1}^{n-1} 2L_i + L_n\right), \text{ where}$$

F = the equivalent in minutes at 121.1°C of all heat at or above 93.3°C, with respect to its capacity to destroy spores or vegetative cells of a particular organism;

θ = the time between successive temperatures (1 minute);

L_o = the lethal rate at the first temperature greater than or equal to 93.3°C;

L_n = the lethal rate at the last temperature greater than or equal to 93.3°C;

L_i = the intermediary lethal rates.

By comparing the F values the degree of uniformity of heat penetration between pouches of potatoes can be observed (see Table 1).

TABLE 1

F_o value at the center of potatoes processed in evacuated pouches in the retort

Processing Time (min)	Size-- Depth & Width (in)	Weight (gm)	F value	Maximum Temperature Reached at the Center of the Potato (°C)
20	1-6/16 x 1-8/16	not available	6.857 8.269 8.685 8.666	119.2 119.8 119.9 120.1
20	1-6/16 x 1-9/16	67.82 62.17	10.876 8.949	120.9 120.2
20	1-6/16 x 1-7/16	60.23 62.10 69.10 68.66 72.18	10.788 13.598 10.574 8.988 10.505	120.5 121.0 120.5 119.9 121.0
12	1-6/16 x 1-8/16	81.84 77.24 79.03 73.78	3.200 3.655 3.207 4.277	118.0 118.6 118.0 119.0

The z value used for determining the lethal rates for the uniformity of heat penetration between pouches which in turn was used to calculate the F value was 10°C (18°F). This value of z was used because it was considered to be the value likely to be found for naturally contaminating micro-organisms on potatoes.

A regression analysis was made on the computer using the Regression-Residual-Plot (RRP) program (see Appendix) between the F_o values and the maximum temperature reached at the center of the potato as listed in Table 1. The data for the two analyses were treated separately according to the processing times, 12 and 20 minutes (see Table 2 for the regression analyses and Table 3 for the comparison of the observed and estimated dependent variables and the % error). The data are illustrated in Fig. 6 and 7. However, it is important to note

TABLE 2

Regression analyses of the relationship between the F_o value (y) and the maximum temperature reached at the center of the potato (x) at 121.1°C for different times

The Best Least Squares Fit of $y = A + Bx$

	Processing Time 20 (min)	12 (min)
A	-331.9	-117.2
B	2.84	1.02
F value (statistical value)	37.21**	58.10*
Degrees of Freedom: regression	1	1
Degrees of Freedom: error	9	2
F_{α} 5%	5.12	18.51
F_{α} 1%	10.56	98.50
Square of the Multiple Correlation Coefficient (R^2)	0.805	0.967

* significant at the 5% level only
** significant at the 1% and 5% levels

TABLE 3

Comparison of F_o values (observed and estimated from the RRP program including the % error that the observed variable varies from the estimated variable) vs the maximum temperature reached at the center of the potato processed at 121.1°C for different times

Processing Time (min) = 20

Maximum Temperature (°C)	Dependent Variable Values (F_o Value) Observed	Estimated	% Error
119.2	6.857	6.658	2.90
119.8	8.269	8.362	1.13
119.9	8.685	8.646	0.44
120.1	8.666	9.214	6.33
120.9	10.876	11.486	5.61
120.2	8.949	9.498	6.14
120.5	10.788	10.350	4.06
121.0	13.598	11.770	13.44
120.5	10.574	10.350	2.11
119.9	8.988	8.646	3.80
121.0	10.505	11.770	12.05

Processing Time (min) = 12

Maximum Temperature (°C)	Dependent Variable Values (F_o Value) Observed	Estimated	% Error
118.0	3.200	3.177	0.73
118.6	3.655	3.789	3.66
118.0	3.207	3.177	0.94
119.0	4.277	4.197	1.88

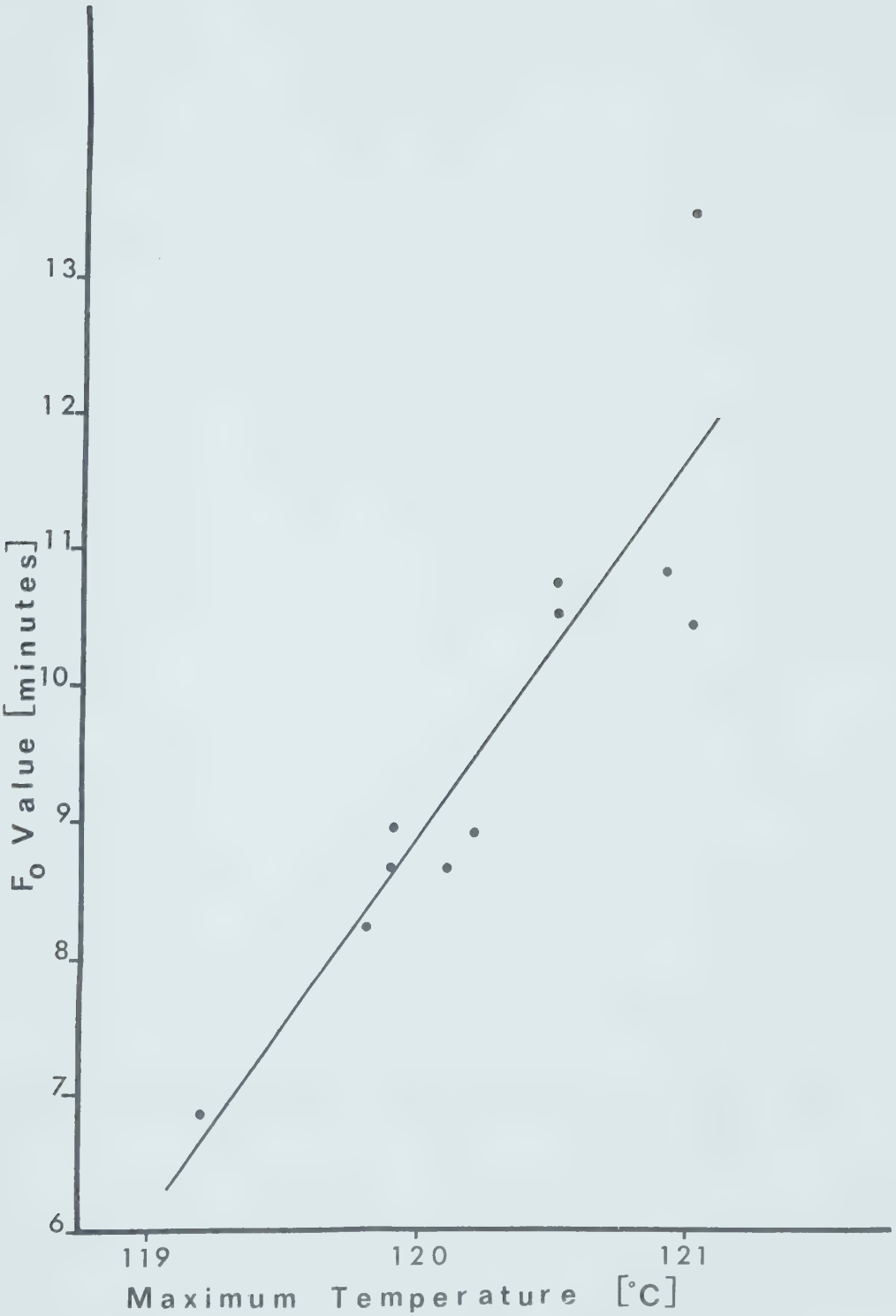

Fig.6 Regression plot of the F_o value versus maximum temperature at the center of the potato for a processing time of 20 min. at 121°C

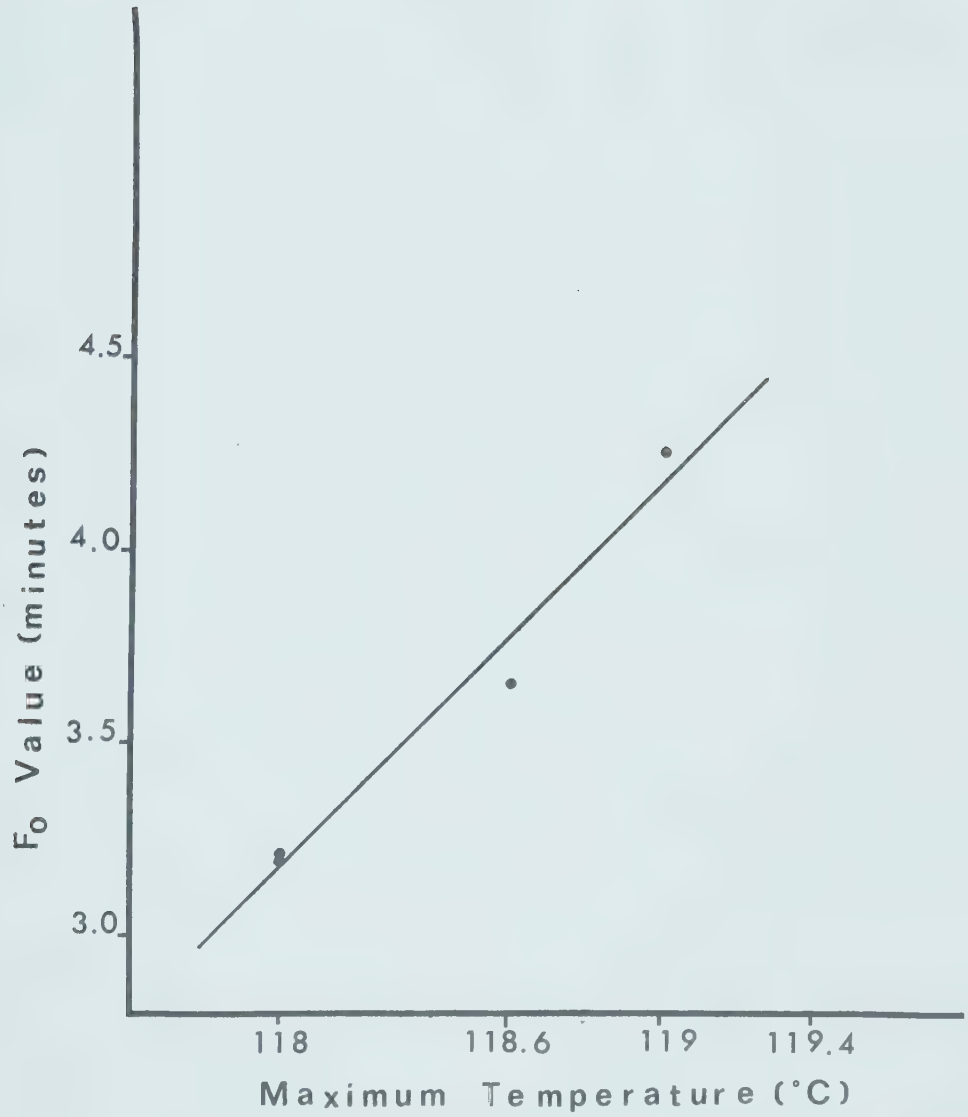

Fig.7 Regression plot of the F_o value versus maximum temperature at the centre of the potato for a processing time of 12 min. at 121°C.

that the processing time referred to here is the time the retort is at 121.1°C. Therefore this is not the time of the lethal heating range and cooling range between 93.3°C and 121.1°C.

In the regression analysis (Fig. 6 and Table 2) that was done, the F_o value (lethality) was related to the maximum temperature reached at the center of the potato for the 20 minute processing time. The correlation between these two appears to be significant at the 5% and 1% levels based on the F value (statistical F). This indicates that the F_o value was markedly affected by the maximum temperature reached at the center of the potato. On the basis of the coefficient of multiple determination (R^2) or the square of the multiple correlation coefficient, which is 0.805, there is an 80.5% variation in the F_o value which is associated with differences in the maximum temperature reached at the center of the potato and 19.5% of the variation is accounted for by other factors, such as size, weight, etc.

For the 12 minute run (Table 3 and Fig. 7) the linear regression is significant at the 5% level but not at the 1% level presumably because of the smaller number of observations. The coefficient of multiple determination with a value of 0.967 implies that 96.7% of the variation in the F_o value (total lethality) is associated with differences in the maximum temperature reached at the cold spot (center of the potato) and 3.3% accounted for by other factors.

For comparative purposes and to determine any alternative effects or interaction that could influence the F value (total lethality), a comparison was made between processing time, F value and the maximum temperature reached at the center of the potato by a multiple regression analysis on the data presented in Table 4. The regression analysis was

TABLE 4

Comparison of F_o value with the variables, processing time and the maximum temperature reached at the center of the potato. Both observed and estimated values for F_o and the % error are given from the regression analysis.

Processing Time (min)	Maximum Temperature Reached at Center (°C)	Dependent Variable Values (F_o Value) Observed	Estimated	% Error
20	119.2	6.857	7.013	2.27
20	119.8	8.269	8.519	3.02
20	119.9	8.685	8.770	0.98
20	120.1	8.666	9.272	6.99
20	120.9	10.876	11.279	3.70
20	120.2	8.949	9.522	6.41
20	120.5	10.788	10.275	4.75
20	121.0	13.598	11.530	15.21
20	120.5	10.574	10.275	2.82
20	119.9	8.988	8.770	2.43
20	121.0	10.505	11.530	9.76
12	118.0	3.200	2.581	19.34
12	118.6	3.655	4.087	11.81
12	118.0	3.207	2.581	19.52
12	119.0	4.277	5.090	19.02

slightly different from the one used previously, as it gave the best least squares fit of the function $F = A + B\theta + CT_M$ where θ refers to time and T_M refers to the maximum temperature reached at the center of the potato (see Appendix). To corroborate the data from the two regression analyses, a stepwise regression was next done where the essential difference from the simple and multiple regression analysis was that the independent variables (time and temperature) are entered into the regression function in stepwise order. The advantage of this regression program was that the proportion of variation of the dependent variable (F_o value) accounted for by each independent variable was given.

The data for both regression analyses are given in Table 5, and the variation (% error) that the observed variable differs from the estimated one is given in Table 4. The estimated values will be different from those previously determined in Table 3 because there are two variables taken into account that are affecting the F value (processing time and the maximum temperature reached at the center of the potato).

TABLE 5

Regression analyses for the comparison of processing time, maximum temperature reached at the center of a potato and the F value for potatoes in evacuated flexible pouches heated in the retort at 121.1°C.

The Best Least Squares Fit of $F = A + B\theta + CT_M$

	Simple and Multiple Regression	Stepwise Regression
A	-295.6	-295.6
B(time)	0.177	0.177
C(temperature)	2.51	2.51
T value for time	1.54	1.54
T value for temperature	6.00	6.00
Degrees of freedom for T	12	12
T critical at 5% (two tailed)	2.179	2.179
F value	97.44**	97.44**
Degrees of freedom: regression	2	2
Degrees of freedom: error	12	12
F_α 5%	3.89	3.89
F_α 1%	6.93	6.93
Square of the multiple correlation coefficient (R^2): time	-	0.012
R^2 for temperature	-	0.930
R^2 for both variables or total	0.942	0.942

* statistical value
** significant at the 5% and 1% levels

From the data given in Table 5 for the regression analyses of the F_o value vs processing time and maximum temperature, the T value for the temperature was significant, but the T value for time was not significant. This was supported by the coefficient of multiple determination (R^2) which shows that 93% of the variation in the F_o value was accounted for by the maximum temperature reached at the center of the potato and only 1.2% was accounted for by the processing time to give a total of 94.2% due to these two factors. In other words, with this retort, time was a more easily controllable variable than temperature reached at the center of the potato. However, if the time that the temperature was in the lethal range (93.3°C to 121 to 93.3°C) had been used instead of the processing time the coefficient of multiple determination for time would probably have been larger. This information is further substantiated by the previous regression analysis (Tables 2 and 3, Fig. 6 and 7), which also had high R^2 values of 0.80 and 0.97 for the maximum temperature reached at the center of the potato for processing times of 20 minutes and 12 minutes respectively. Thus, even though there was a large difference in the F_o values calculated for the 20 minute and 12 minute processing times the coefficient of multiple determination does not indicate that there is this difference. The lower F_o values at 12 minutes are because the maximum temperature reached at the center is lower and because the length of time that the center is in the lethal range is shorter even though the data presented does not show this. The F value (statistic) from Table 5 was highly significant, but was probably mostly controlled by one factor, the maximum temperature reached at the center of the potato, as depicted by the high value for the coefficient of determination.

In the table in the Appendix, the equivalent time at 121.1 °C was calculated, that is, by using the lethal rate (L_i) for the maximum temperature reached at the center of the potato and dividing the F_o value (total lethal rate) obtained by this value (L_i), the equivalent time at 121.1 °C can be calculated. This is the time, at the maximum temperature reached, that the F_o value is equivalent to, if there was no heating and cooling periods such that the center of the potato could be immediately at the maximum temperature reached at the center of the potato without a lag period when heating, and cooled immediately below 93.3 °C without a lag period at the end of the heating cycle. By comparing these times the differences between the processing time of 20 minutes and 12 minutes can readily be seen. For example, at a processing time of 12 minutes the F_o value of 4.277 is equivalent to 6.9 minutes at 119 °C, and for a 20 minute processing time the F_o of 6.857 is equivalent to 10.7 minutes at 119.2 °C. Thus, because of the difference in time, even though the maximum temperature reached at the center is approximately the same, the F_o values are largely different, because of the difference in effective time, or the time that the temperature at the center is in the lethal range (greater than 93.3 °C). Although the above analyses confirm what would appear to be obvious, it was deemed necessary to have proof of this by doing the statistical analyses.

The analyses above do not explain the apparent discrepancy in Table 1, in the third run of 20 minutes, where the maximum temperature of 121.0 °C is reached on two occasions, but in one pouch an F_o value of 13.6 is achieved while the other is only 10.5. The reason for this discrepancy is because even though the same maximum temperature was reached, it was not reached at the same time, and the data in Table 1

does not show this. However, the difference in equivalent time of 13.6 vs 10.5 minutes at 121°C is obtained from the Table in the Appendix. Therefore, the higher F value of 13.6 is probably because the potato in question was lighter and the maximum temperature of 121°C was reached before the same maximum temperature was achieved in the heavier potato, and as there was a longer time at the same temperature a higher F_o value was obtained. Thus, the F_o value, although markedly affected by the maximum temperature reached at the center of the potato for a constant processing time, is also influenced by the length of time the center is at this maximum temperature, or the length of time that the center of the potato is in the lethal range which is above 93.3°C. The longer the center is at the maximum temperature and in the lethal range, the greater will be the value of F_o. Similarly, the higher the maximum temperature at the center of the potato, the higher the lethal value and thus the higher is the value of F_o, which is substantiated by the value for the square of the multiple correlation coefficient (0.93) for this independent variable (Table 5).

B. Determination of the Number of Naturally Occurring Micro-organisms on the Surface of Peeled Potatoes

Hand-peeled and commercially peeled potatoes were tested for the number of heat resistant spore-forming micro-organisms naturally present. The commercially peeled potatoes (caustic peeled) were already treated with sodium metabisulphite (0.5% w/v) to reduce browning and to neutralize the lye. The hand-peeled potatoes were dipped in a 1.7% (w/v) sodium bisulphite bath for 90 sec to inhibit browning. The desired concentration of the bisulphite dip was determined previously by Mrs. B. Burtnick (pers. comm.) and although it was not the minimum concentration that cleared browning overnight after processing, it only left an average residue of 50 ppm and was rated as third choice out of 8 dips examined by a taste panel. The first two choices were dips composed of bisulphite and citric acid, and bisulphite and sodium acid pyrophosphate. As the first two choices both included bisulphite and the difference between the first three choices was not appreciable the choice of the bisulphite alone at a 1.7% (w/v) concentration was made for further use in experiments. Also the difference in concentration of 1.7% vs 1.4% afforded a margin of safety for potatoes with a higher than normal content of reducing sugars. The large difference between the concentration of the commercial dip and the dip used in this laboratory can be explained by the different heat treatments used after the dip. Processing (at 121.1°C) of the potatoes gives a much more severe heat treatment than the heating given the commercial potatoes, which was either French frying or no heating.

Four potatoes, 1-3/4 in in diameter or less were then placed with sterile forceps into a flexible pouch. Ten ml of sterile peptone

water (0.1% w/v) was then added, before the pouches were sealed under low vacuum. The potatoes in the pouch were washed and palpated with the 10.0 ml of peptone water by the vibration of a Vortex mixer for one to two minutes. Then 5.0 ml of the peptone rinse was removed and put into a sterile test tube. This was heated for 10 minutes at 80°C to destroy vegetative cells, and then quickly cooled in an ice bath. Dilutions from this heat-treated rinse were plated out in triplicate using plate count agar for aerobes and reinforced Clostridia agar for anaerobes, and were incubated at 37°C (see Table 6).

The counts of the natural level of contamination, a total of both aerobes and anaerobes varied from a total of 9×10^1 colonies/pouch to 1.4×10^4 for the commercially peeled potatoes with an average of 3.9×10^3. The average of the unsulphited commercially peeled potatoes was 9.7×10^3 colonies/pouch which is higher than the average for the commercially peeled sulphite treated potatoes. Although sodium metabisulphite is primarily used as a deterrent for the browning of potatoes it would appear to have some bacteriostatic effect. However, to determine whether it is appreciably bacteriostatic or not, further examinations of the natural level of contamination on commercially peeled unsulphited potatoes would be necessary. The average of the total counts of the hand-peeled potatoes was found to be 3.3×10^4 spores/pouch which is higher than the average of the commercially peeled potatoes, 3.9×10^3 spores/pouch. However, this is expected because the commercially peeled potatoes which are peeled by the use of lye should have slightly lower counts due to the bactericidal effect of lye, whereas for hand-peeled potatoes the only bactericidal effect would be that of dipping in the sodium bisulphite solution (1.7% w/v) for 90 sec which leaves a residue

TABLE 6

Natural level of heat-resistant spore-forming micro-organisms on peeled potatoes

	colonies/pouch (4 potatoes)		
	Aerobic	Anaerobic	Total
Commercially Peeled			
Bisulphite treated	1.4×10^2	1.4×10^2	2.8×10^2
	1.8×10^2	1.2×10^2	3.0×10^2
	1.9×10^2	5.5×10^1	2.4×10^2
	1.7×10^2	7.5×10^1	2.4×10^2
	1.0×10^1	1.2×10^2	1.3×10^2
	4.0×10^1	5.0×10^1	9.0×10^1
	1.5×10^3	1.0×10^1	1.5×10^3
	1.0×10^2	1.5×10^2	2.5×10^2
	1.5×10^3	1.8×10^2	1.7×10^3
	2.7×10^3	4.2×10^3	6.9×10^3
	4.5×10^3	3.9×10^3	8.4×10^3
	5.2×10^3	5.8×10^3	1.1×10^4
	7.6×10^3	6.8×10^3	1.4×10^4
	4.4×10^3	7.0×10^3	1.1×10^4
Average	2.0×10^3	2.0×10^3	3.9×10^3
No bisulphite treatment	4.6×10^3	4.8×10^3	9.4×10^3
	7.0×10^3	3.1×10^3	1.1×10^4
Average	5.8×10^3	4.0×10^3	9.7×10^3
Hand-peeled			
Bisulphite treated	9.0×10^3	3.6×10^2	9.4×10^3
	5.1×10^4	4.8×10^3	5.6×10^4
Average	3.0×10^3	2.8×10^3	3.3×10^4

of only 50 ppm.

Fuller, El-Bisi and Francis (1965) found that the aerobic counts on pre-peeled French fry cut potatoes were as follows:

Hand-peeled 3.7×10^2/600 g package

Commercially prepared 7.4×10^3/600 g package.

From Table 6 the aerobic counts on commercially peeled small whole potatoes was 2×10^3 spores/pouch for 4 potatoes/pouch. The average weight of potatoes in the pouches tested here was about 300 g. Accordingly, the count of aerobic heat resistant spores determined in this work compared favorably with that obtained by Fuller et al. The results of both aerobic and anaerobic spores given by Fuller et al., 9.6×10^3/600 g package is comparable to 4.8×10^3/1/2 package. This figure can be compared to the average total count of 3.9×10^3/pouch obtained for the commercially peeled potatoes in this laboratory. This would seem to indicate that the technique used compares favorably to the one Fuller et al. used which differed by the fact that 200 ml of sterile distilled water was added to a package instead of 10 ml, resealed and shaken vigorously for 5 minutes instead of palpation for 2 minutes on the Vortex mixer, before an aliquot was removed for plating.

Although Fuller et al. do not give the type of peeling used for the commercially peeled potatoes, it probably was not steam because information from Thorpe (1973) was that steam peeled potatoes gave 0 - 1 spore/potato which is considerably less than that found by Fuller et al. for commercially peeled potatoes or for this lab for caustic peeled potatoes. Thus, this would seem to indicate that the method of peeling influences the level of natural contamination, which in turn

determines the severity of heat treatment required to produce a microbiologically stable product.

C. Search for a Spore-forming Bacterium of Suitable Heat Resistance

The production of a sufficient number of heat-resistant spores for the test inoculation of pouches of potatoes involved study of three organisms, Clostridium sporogenes (P.A. 3679), Cl. thermosaccharolyticum (7956), and Bacillus stearothermophilus (B7/202). Initially, it was considered desirable to use an anaerobic spore-forming micro-organism, because the pouches of potatoes were evacuated before retorting and anaerobic conditions were maintained in the pouches. However, as indicated below, difficulties were encountered with the anaerobic cultures and since the initial search for suitable times and temperatures of treatment merely involved the determination of numbers of surviving spores and not their subsequent growth in pouches of potatoes, it was realized that an aerobic or facultative anaerobic spore-former would be quite suitable for this purpose; hence the use of B. stearothermophilus.

Initial work was done with P.A. 3679 (Canada Packers, Toronto). This involved growing the culture in liquid Oxoid Reinforced Clostridial Medium (RCM) which had been reported to produce a heavy spore crop in 3 days at 37°C (Futter and Richardson, 1970). The stock culture became contaminated and only grew poorly in RCM. A new medium, supplemented trypticase broth (King and Gould, 1971) containing (w/v) trypticase 4% (BBL), yeast extract 0.15% (Difco), ammonium sulphate 0.15%, L-ascorbic acid 0.05%, L-cysteine 0.025%, with a pH of 7.2 was next tried to obtain

better spore production and more rapid growth and this proved to be successful. Before the culture could be satisfactorily purified fresh cultures of P.A. 3679 were obtained from Canada Packers and the National Department of Health and Welfare. These were grown up in the supplemental trypticase broth and tested for heat resistance using sealed glass ampoules (2 ml) in an oil bath. Both cultures were not sufficiently heat resistant having D_{100} values of 3.9 and less than 2.6, whereas a minimum D_{121} value of 3.5 was required in order to be able to obtain easily a stock culture of 10^7 spores/ml and still have survivors after 20 - 25 minutes of processing at 121°, because our preliminary data indicated that an $F_o = 3$ was achieved at the center of a 1-3/4 in potato only after this period of processing.

As thermophilic spores are normally more heat resistant than mesophilic spores, cultures of Cl. thermosaccharolyticum and B. stearothermophilus were next investigated.

Freeze dried cultures of B. stearothermophilus (8157) were inoculated into flasks of nutrient broth and incubated at 55°C. When no growth occurred, the flasks were aerated and later the temperature was lowered, but still no growth occurred. In the meantime, a culture of B. stearothermophilus (B7/202) was received from the National Institute for Research in Dairying (Shinfield, Reading, England). These spores in aqueous suspension were inoculated directly into Roux flasks containing supplemented nutrient agar which was known to support the growth of this culture (Finley and Fields, 1962). Within a week there was a profuse growth of spores which was recovered by using sterile distilled water and sterile glass 'hockey sticks'. The spores were centrifuged at 16,000 rpm and washed several times with sterile distilled

water. This suspension was tested for heat-resistance, as above, and proved to be satisfactory having a D_{121} value of 3.6.

Results with Cl. thermosaccharolyticum after inoculating a freeze-dried culture into sterile pea broth (Folinazzo and Troy, 1954) were unfavorable as difficulty was experienced in obtaining a satisfactory crop of spores. Before this difficulty was resolved a suitable heat resistant suspension of B. stearothermophilus spores was produced.

D. Determination of the Optimum Growth Temperature for Heated B. stearothermophilus Spores

Any estimation of the effectiveness of heat treatment in the destruction of bacterial spores usually depends on a measurement of the viability of the survivors. This is usually done by a cultural procedure involving a colony count. Although there is little information available on the effect of incubation temperature on the recovery of heat treated bacterial spores; the results available suggest that some species recover better at temperatures below the optimum for that of unheated spores. Edwards et al. (1965) showed that B. subtilus spores subjected to 'ultra high temperature' treatment (150°C/2 sec) showed greater recovery at 32°C than at 45°C; which was opposite to the results they obtained for unheated spores, and Prentice and Clegg (1974) found an optimum recovery temperature of c. 30°C for heated B. subtilus spores whereas unheated spores grew equally well at 16 - 48°C. Futter and Richardson (1970) found heat damaged spores of Cl. welchii gave optimum recovery at 27°C as did Williams and Reed (1942). Cook and Gilbert (1968) obtained maximum colony counts for B. stearothermophilus spores heated at 115°C for various times on incubation at 45 - 50°C whereas unheated

spores had a maximum recovery at 50 - 65°C. Because heat treated spores appeared to be affected by the temperature at which they are recovered the optimum growth temperature was determined for B. stearothermophilus spores recovered from the surface of heat treated potatoes.

The potatoes were peeled, placed in pouches, inoculated with c. 5×10^7 B. stearothermophilus spores/pouch, vacuum sealed and processed in the retort at 121.1°C for 20 minutes. Serial dilutions of the heat treated spores were made in 9 ml amounts of 0.1% (w/v) of Bacto-Peptone (Difco) after making the initial dilution by adding 9 ml of peptone water to the pouch to wash off the spores and palpating with the aid of the Vortex mixer. Then 0.2 ml from a suitable dilution was added to 4.5 ml of the molten medium (supplemented nutrient agar) in which the concentration of agar had been increased to 5% (w/v) at 45° in roll tubes (Clegg and Sherwood, 1947). The tubes were cooled under running water while being rotated on an Astell roller (Astell and Co., Brownhill Road, London) so that the medium formed a thin film around the inside of the tubes which were then placed upright in the gradient temperature incubator (Packer, Prentice and Clegg, 1973). The temperature gradient incubator was heated and cooled by circulating liquids and had a linear temperature gradient with the cold end set at c. 44°C and the hot end at c. 65°C. Temperatures for each of the tubes, each in an individually insulated sample well, at the various positions in the gradient bar were determined by measuring the temperature of water in a corresponding sample well.

Packer, Prentice and Clegg (1973) did calculations to determine the deviations from linearity from the hot to cold end of the incubator. The maximum deviation from linearity with a temperature gradient aluminum

jacket was calculated to be less than 0.08°C and measurements on an experimental run gave a maximum deviation of 0.13°C. Packer *et al.* also determined that the difference between the hottest and coldest points at the bottom of the sample wells between consecutive rows was less than 2%.

Three separate runs were done, due to the limitation of space in any 1 row (that is 1 temperature) in the gradient temperature incubator. There were only 6 sample wells/row to hold 2 or 3 dilutions with 2 replicates and 1 sample well to test for temperature. There was one run done for the unheated spores and two runs of the heated spores. However, all three runs had approximately the same size of inoculum (*c.* 5×10^7 spores/pouch). The size of inoculum was not critically controlled and seemed relatively unimportant in comparison to the degree of variation within a run between temperatures. For the unheated spores after the 9 ml of peptone water was added to the pouch, 5 ml was removed and placed in a sterile test tube in order to heat it for 10 minutes at 80°C to destroy any vegetative cells.

The colonies formed were counted every 24 hours for 3 days after which no increase in colony count was observed even at the lowest temperature. The highest colony count for each tube was taken as the actual count.

The concentration of agar was increased to 5% (w/v) to compensate for the dilution of the medium by the inoculum and to reduce melting experienced at the higher temperatures (54° - 65°C) whereby the thin film of media became a molten layer of liquid at the bottom of the roll tube.

The colony count data as affected by incubation temperature is listed in Table 7 and illustrated in Fig. 8 and 9. The results of the

TABLE 7

The effect of incubation temperature on the recovery of spores of B. stearothermophilus after being heated for 20 minutes at 121.1°C

(Averages of duplicate roll tubes)

Temperature (°C)	Unheated Spores (colonies/ml)	Heated Spores	
		Run I	Run II
44	6.25×10^7	5.0×10^3	7.5×10^4
47	8.9×10^7	6.0×10^3	8.8×10^4
51	7.5×10^7	2.3×10^3	4.6×10^4
54	1.15×10^8	7.0×10^3	3.8×10^4
57	4.25×10^7	5.8×10^3	1.4×10^5
61	2.1×10^7	5.5×10^3	7.5×10^4
65	8.5×10^6	3.8×10^2	1.1×10^5

individual dilutions aregiven in the Appendix.

In Fig. 9 for the heated spores, the counts in Run I drop down markedly at the 65°C temperature. How acceptable this data is can be seen from the Table in the Appendix. As the colony counts for this temperature were below the accepted range of 15 - 150 colonies/roll tube, this point on the graph is suspect but it does follow the trend of the unheated spore curve (Fig. 8). However, once again for the unheated spores, only one dilution of counts was used, because only the one dilution fell into the accepted range. This tends to markedly shift the curve one way, especially when more than one point is affected. Thus, the variability in colony counts between dilutions can markedly affect the curve drawn.

One reason for the discrepancies shown in the Table in the Appendix for the heated spores is that there may be sufficent heat damage done (at 121.1°C for 20 minutes) that there are many spores

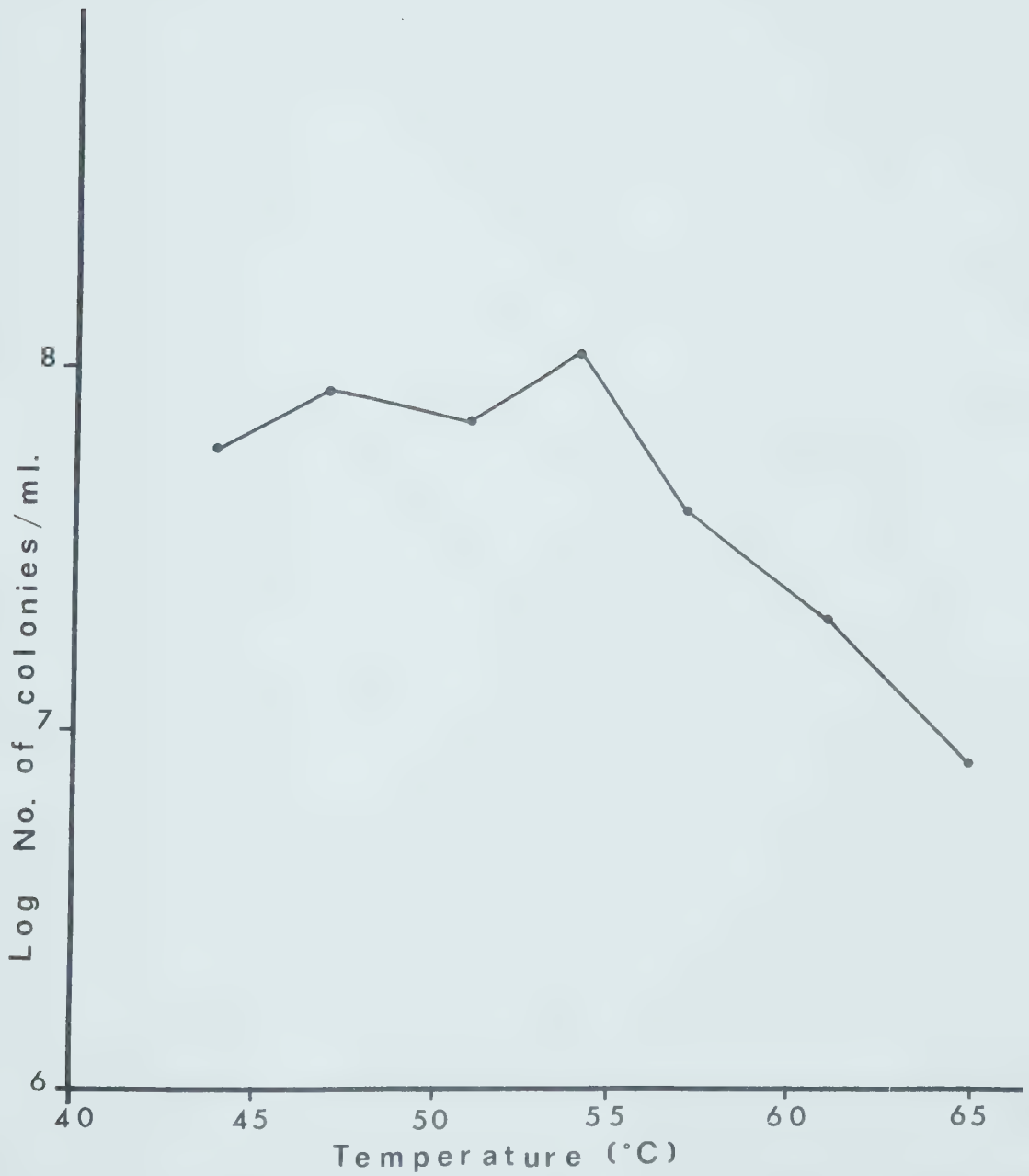

Fig.8 The effect of different incubation temperatures on the growth in supplemented nutrient agar of unheated B.stearothermophilus spores inoculated onto potatoes in a pouch

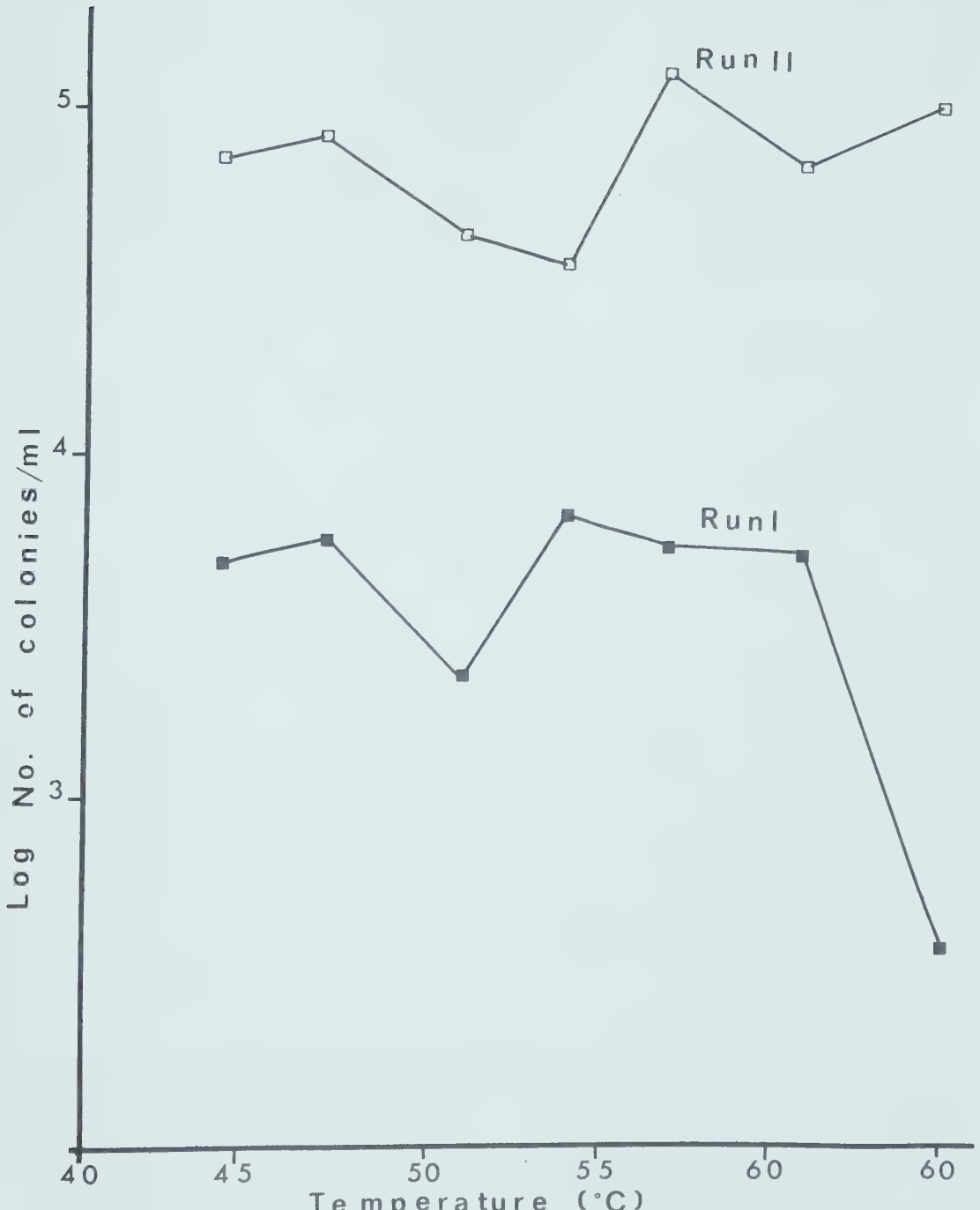

Fig. 9 The effect of different incubation temperatures on the recovery in supplemented nutrient agar of B. stearothermophilus spores after heating at 121°C for 20 min. in sealed pouches of potatoes.

which do not recover and this results in a large variation between counts for the same dilution or between dilutions from the same initial inoculum. However, the unheated spores also have discrepancies within and between dilutions, with comparatively no heat damage. This could be explained by the starch granules washing off the potatoes into the dilution tubes and interfering with the distribution of spores within the dilution tube, which in turn affects the distribution in subsequent dilutions and when pipetting.

From Fig. 9 the optimum recovery temperature for heated spores can be deduced. The unheated spores (Fig. 8) do not seem to have a depression in counts in the range of 50 - 55°C, whereas with the heated spores both curves have an apparent depression in this range. Therefore, the optimum temperature for recovery was chosen to be 47°C, even though there would appear to have been equal justification for choosing a higher temperature (57°C) at which the results also peaked, but on the basis that heat damaged micro-organisms require lower temperatures for recovery, the lower incubation temperature was chosen. Also the counts at the lower temperature were at least as great as in the higher ranges and there were fewer difficulties with media during incubation at this lower temperature.

E. Determination of the D and z Values for B. stearothermophilus Spores

The D and z values found in the literature for this micro-organism were deemed inadequate, because the conditions under which these were tested would not have been the same as in the present work. That is, suspended in the water containing dissolved nutrients (starch,

carbohydrates, etc.) on the surface of the potato. Such a solution might have had either a protective or sporicidal effect for spores undergoing heating.

Heat destruction curves for this organism under the conditions of processing were determined using the retort as a laboratory tool and an inoculum of c. 5×10^7 spores/pouch with varied treatment times at 121°C, 115°C and 110°C which would yield surviving spores. The retort was used as a laboratory tool instead of resorting to an oil bath to determine the heat destruction curves because the D value determined would be under different conditions as the heating up and cooling down from processing temperature adds to the lethality of the heat treatment. From the heat destruction curves the decimal reduction time, D, was determined by a regression analysis using the RRP computer program given in the Appendix. The following times and temperatures were tested:

110°C for 40, 60, 100 and 120 minutes

115°C for 20, 40, 68 and 100 minutes

121°C for 5, 10, 12 and 15 minutes

The times used were the processing time, such that the retort was at the specified temperature for the time given. To have used the retort at temperatures higher than 121.1°C would have caused various technical difficulties, i.e. setting of release valve, insurance coverage, etc., so to corroborate the tests using the retort, tests were also conducted by heating spores (1.5 ml of c. 5×10^7 spores/ml) in sealed glass ampoules in an oil bath at 130°C (see Materials and Methods for details of procedure). The time of heat treatment in the oil bath at 130°C was 4, 8, 10 and 12 minutes. The results (D value) using this technique gave

results compatible with those from the retort (see Table 8 and Fig. 12 and 13).

The heat destruction curves were drawn after a regression analysis was made on the data, so as to obtain the best line of fit from which to calculate the decimal reduction time, D. The regression data from the analyses are listed in Table 8 and the plots are illustrated in Fig. 10 to 13.

Once the decimal reduction times for the different temperatures were obtained, the log of the D values could be plotted against temperature to obtain the z value. Therefore a regression analysis was done on the D values against temperature (see Table 9, which is illustrated in Fig. 14). Although the F value (statistical value) from Table 9 is not significant at either the 1% or 5% levels this is because there are only three sets of data available (since the D value determined at 130°C is under different conditions and cannot be included) to plot which gives very low degrees of freedom (1 and 1) which gives very high F_{α} values.

The analyses including the values for the independent variable, and the corresponding observed and estimated dependent variable along with the % error for the determination of the decimal reduction time, D, are given in Table 10. The reciprocal of the slope obtained from the curve of the plotted estimated values gives the D value. The column of % error gives the difference between the observed and estimated values for the dependent variable and shows the variation that each point at a given time varies with the estimated point that gives the best fit of $\log y = A + Bx$.

TABLE 8

Regression analyses using the RRP program to determine the decimal reduction times for B. stearothermophilus spores inoculated onto the surface of potatoes in evacuated pouches after heating at different temperatures in the retort for different times, except for 130°C which had spores heated in ampoules

The Best Least Squares Fit of $\log N^{\dagger} = A + B\theta$

	Processing Temperature (°C)			
	110	115	121	130
A	10.43	8.74	8.56	9.31
B	-0.033	-0.047	-0.157	-0.278
F value*	517.45**	26.78**	232.7**	62.81**
D F for regression***	1	1	1	1
D F for error	10	7	9	9
F_{α} 5%	4.96	5.59	5.12	5.12
F_{α} 1%	10.04	12.25	10.56	10.56
R^2****	0.98	0.79	0.96	0.87
D value = $\frac{-1}{B}$	30.12	21.08	6.36	3.60

† N = number of colonies/ml
* statistical value
** significant at the 1% and 5% levels
*** D F stands for degrees of freedom
**** R^2 stands for the square of the multiple correlation coefficient

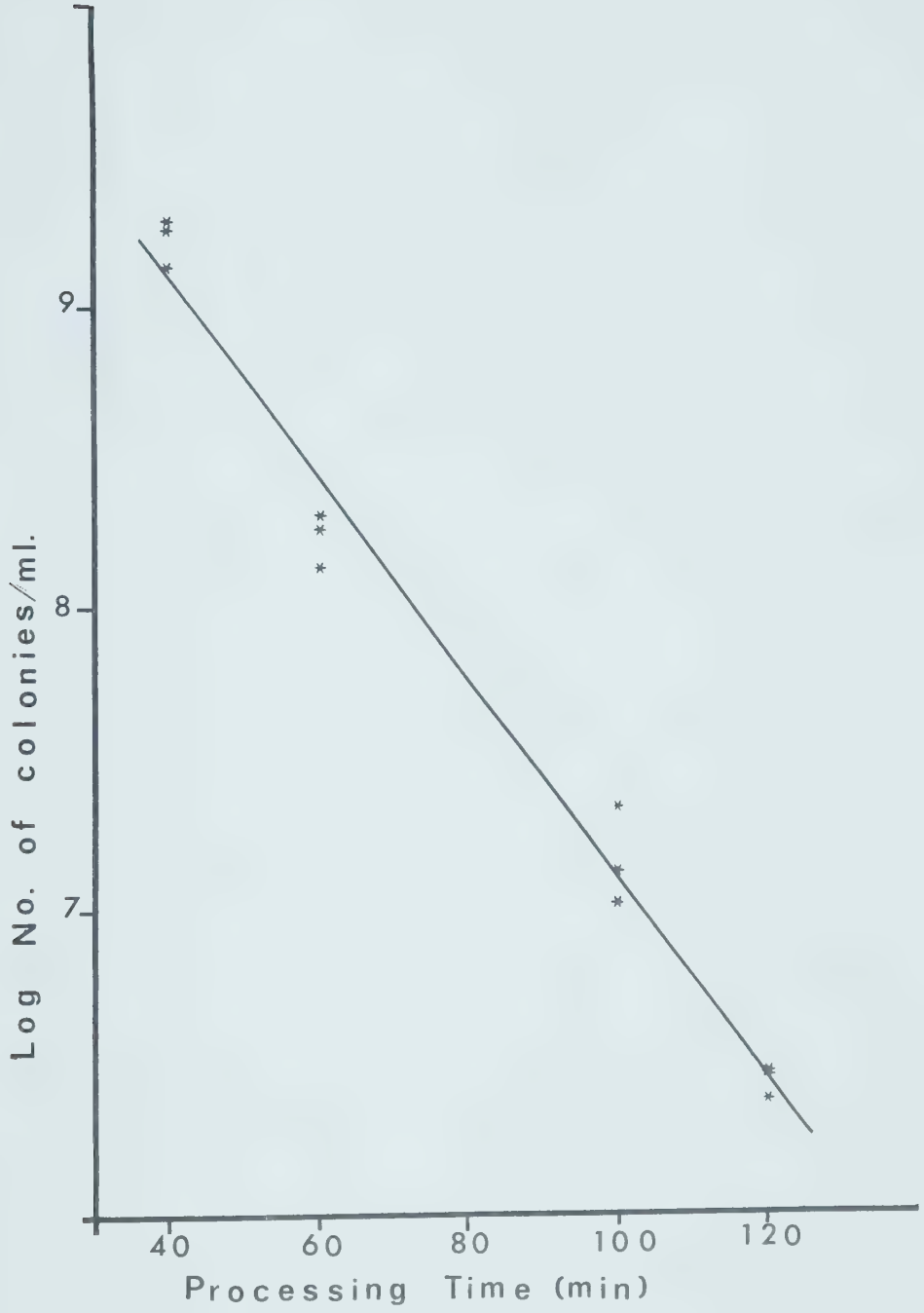

Fig.10 Determination of the D value using a regression plot of the number of survivors of B. stearothermophilus spores inoculated onto the surface of potatoes in evacuated pouches after heating for varied times at 110°C.

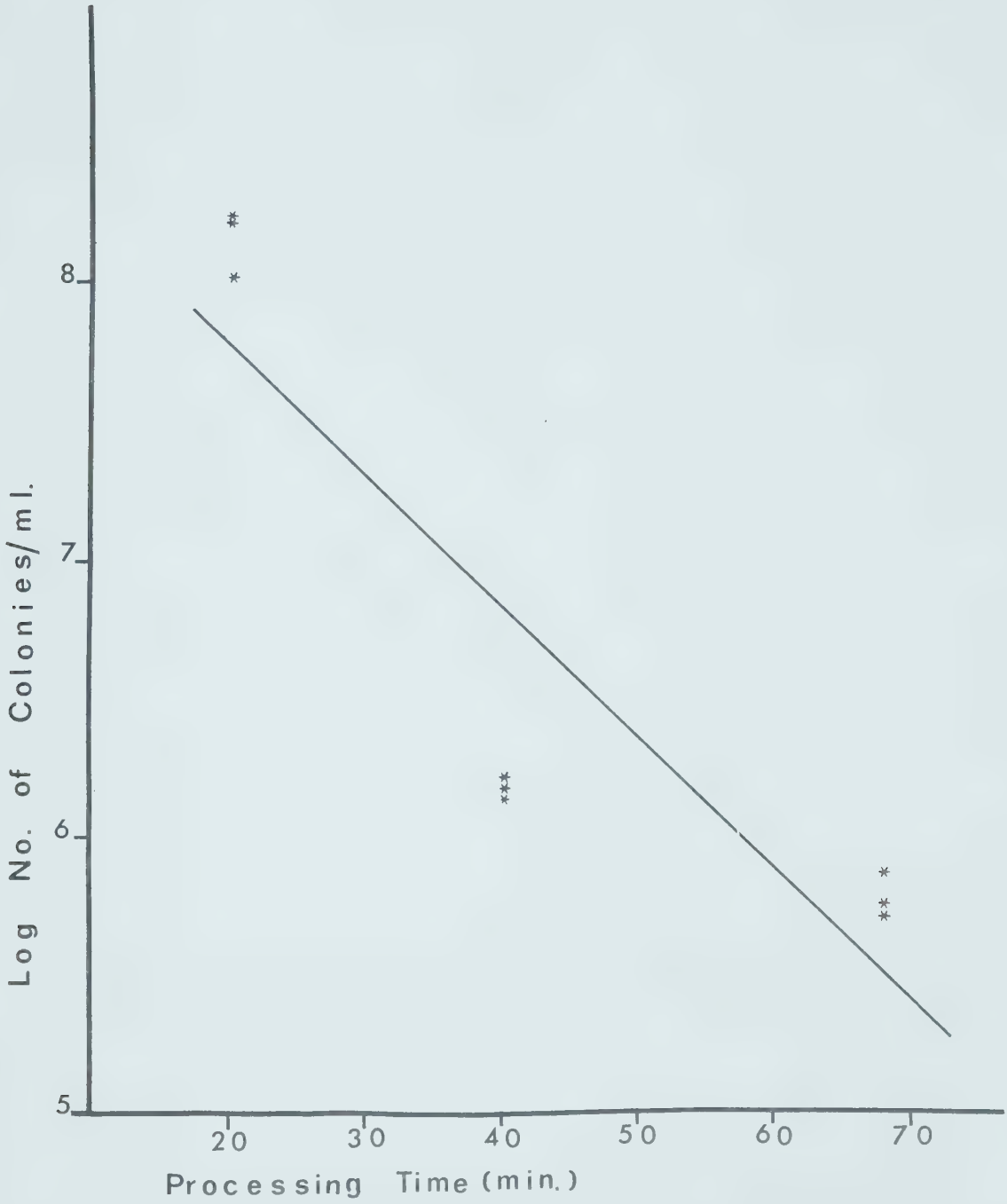

Fig.11 Determination of the D value using a regression plot of the number of survivors of B.stearothermophilus spores inoculated onto the surface of potatoes in evacuated pouches after heating for varied times at 115°C in the retort.

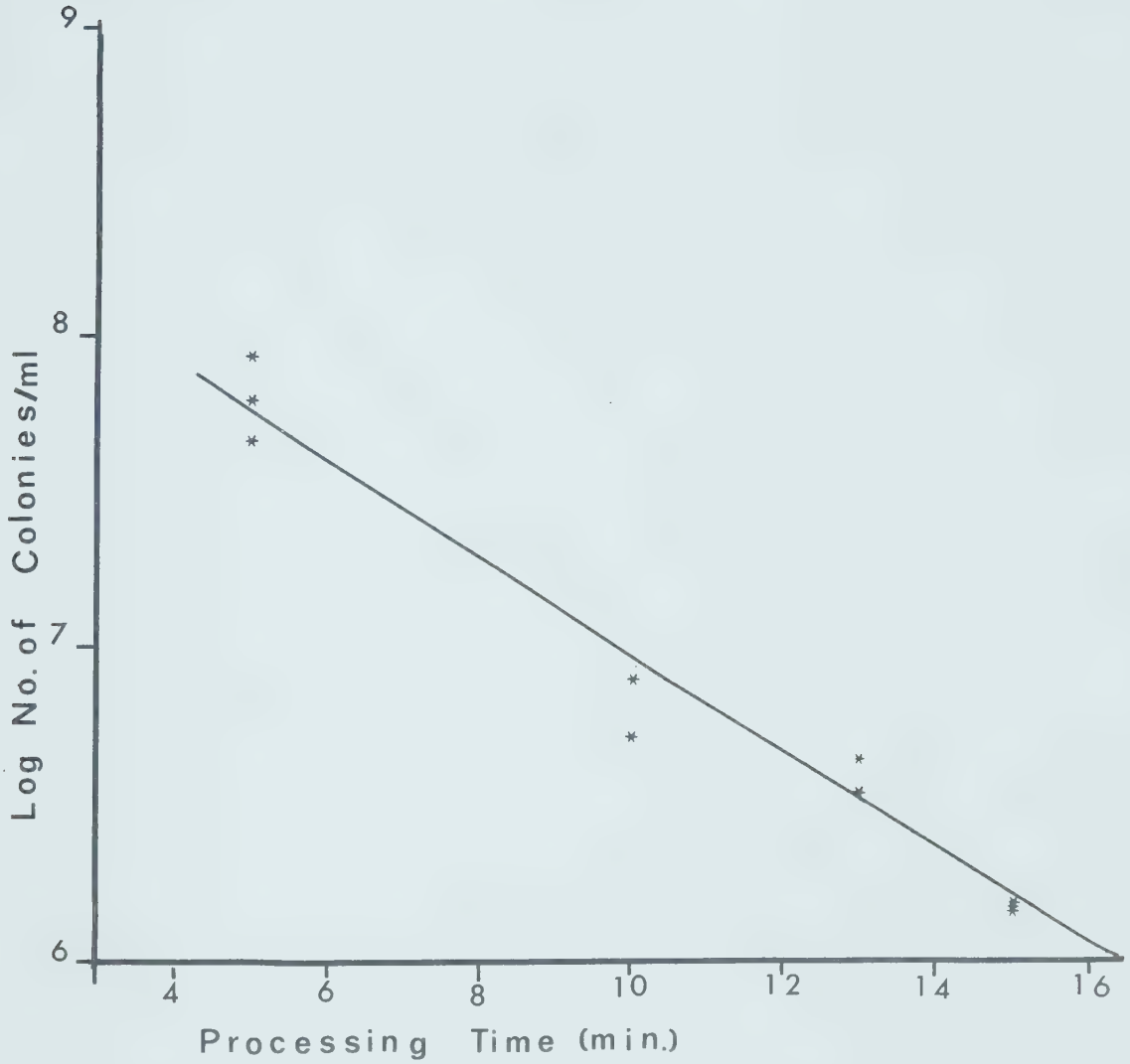

Fig.12 Determination of the D value using a regression plot of the number of survivors of B.stearothermophilus spores inoculated onto the surface of potatoes in evacuated pouches after heating for varied times at 121°C in the retort.

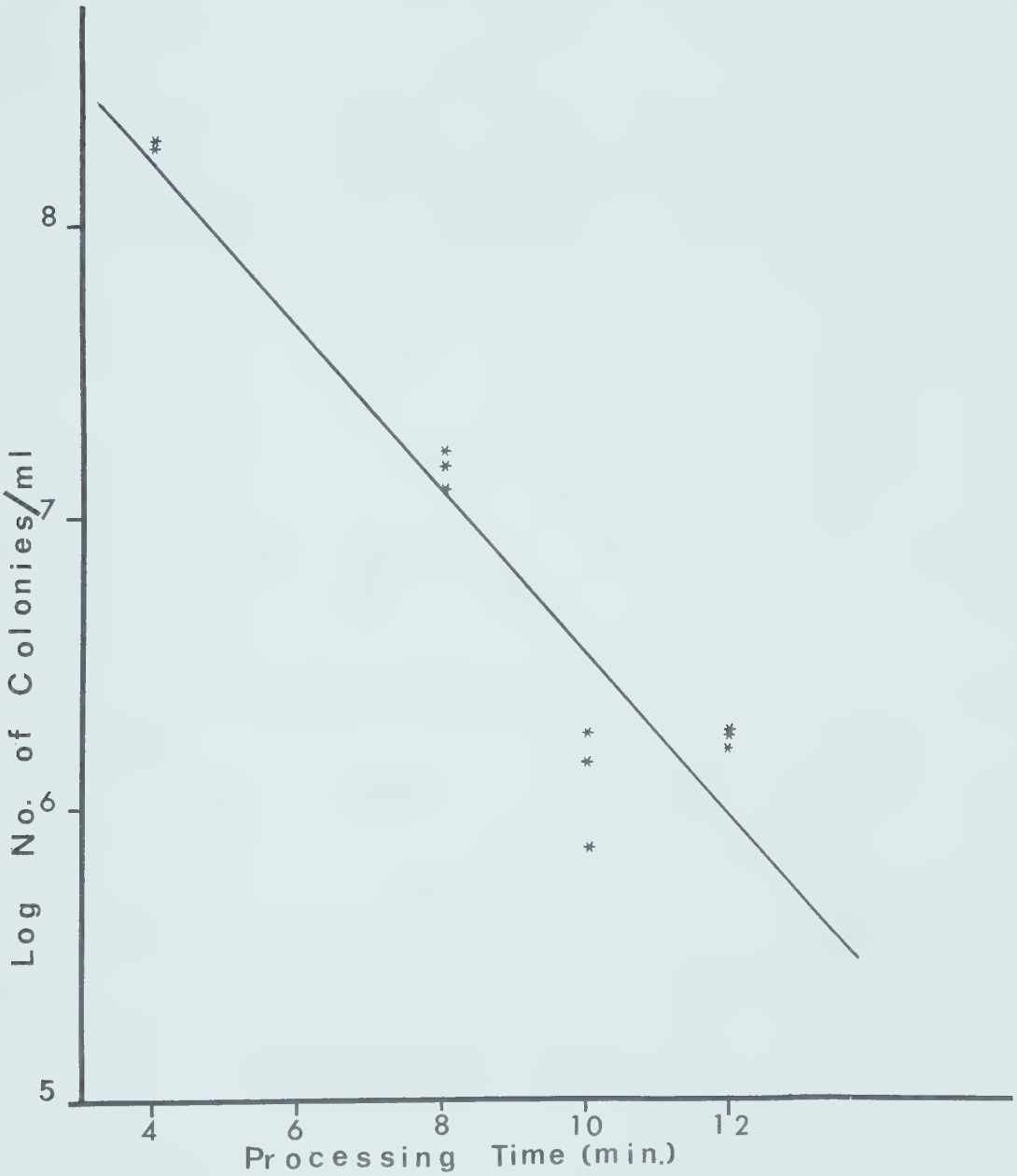

Fig.13 Determination of the D value using a regression plot of number of survivors of B. stearothermophilus spores heated in ampoules for varied times at 130°C in an oil bath.

TABLE 9

Regression analysis using RRP program to determine the z value for B. stearothermophilus spores inoculated onto the surface of potatoes in evacuated pouches which were heated in the retort

The Best Least Squares Fit of $\log D = A + BT$

A		8.38
B		-0.062
F value*		15.26†
Degrees of freedom for regression		1
Degrees of freedom for error		1
R^2: square of the multiple correlation coefficient		0.94
F_α	5%	161.4
	1%	4052
z value (°C) = $\frac{-1}{B}$		16.06

* statistical value
† not significant at the 5% or 1% levels

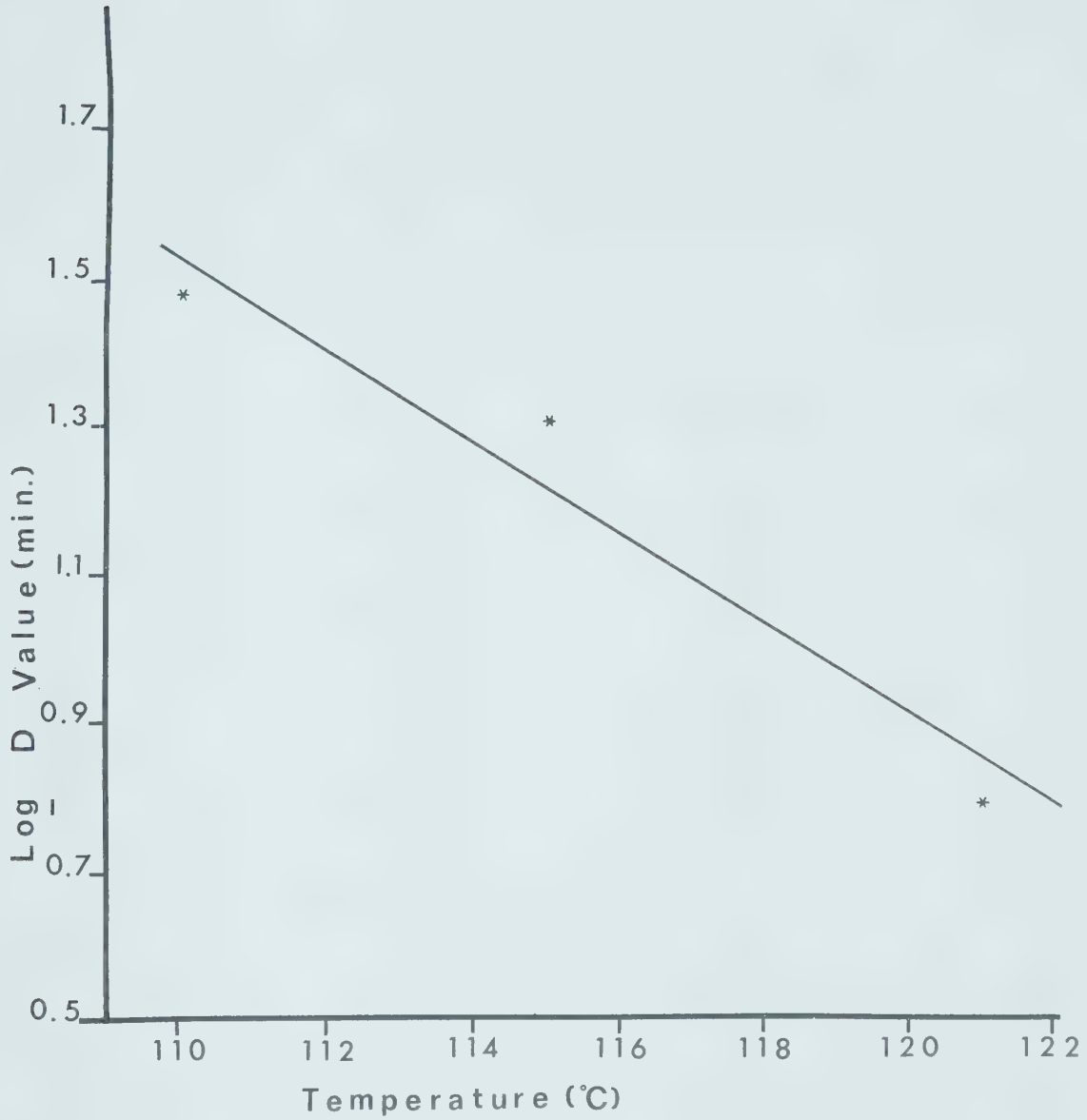

Fig.14 Determination of the z value for *B. stearothermophilus* spores inoculated on the surface of the potatoes heated in a retort using a regression plot of the log decimal reduction time, D, at different temperatures.

TABLE 10

The best least squares fit of log y = A + Bx, using the RRP program for the determination of the decimal reduction time, D, from a plot of log number of survivors of B. stearothermophilus vs time of heating at different temperatures (with the observed and estimated values for the dependent variable and the % error)

Processing Temperature (°C) = 110

Processing Time (min)	Dependent Variable Values (Log colony count/ml) Observed	Estimated	% Error
40	9.1461	9.1026	0.48
40	9.2788	9.1026	1.90
40	9.2553	9.1026	1.65
60	8.1461	8.4386	3.59
60	8.2788	8.4386	1.93
60	8.3222	8.4386	1.40
100	7.0414	7.1107	0.98
100	7.1461	7.1107	0.50
100	7.3617	7.1107	3.41
120	6.3802	6.4468	1.04
120	6.4771	6.4468	0.47
120	6.4624	6.4468	0.24

Processing Temperature (°C) = 115

Processing Time (min)	Dependent Variable Values (Log colony count/ml) Observed	Estimated	% Error
20	8.2355	7.7896	5.41
20	8.0414	7.7896	3.13
20	8.2553	7.7896	5.64
40	6.2227	6.8412	9.94
40	6.1335	6.8412	11.54
40	6.1732	6.8412	10.82
68	5.7324	5.5133	3.82
68	5.7634	5.5133	4.34
68	5.8751	5.5133	6.16

TABLE 10 (Continued)

Processing Temperature (°C) = 121

Processing Time (min)	Dependent Variable Values (Log colony count/ml) Observed	Estimated	% Error
5	7.9494	7.7736	2.21
5	7.8062	7.7736	0.42
5	7.6721	7.7736	1.32
10	6.8976	6.9870	1.30
10	6.7324	6.9870	3.78
13	6.5441	6.5150	0.44
13	6.6628	6.5150	2.22
13	6.6628	6.5150	2.22
15	6.1614	6.2004	0.63
15	6.1818	6.2004	0.30
15	6.1703	6.2004	0.48

Oil Bath Temperature (°C) = 130

Time in Oil Bath (min)	Dependent Variable Values (Log colony count/ml) Observed	Estimated	% Error
4	8.2788	8.2030	0.92
4	8.3010	8.2030	1.18
8	7.2041	7.0930	1.54
8	7.2553	7.0930	2.24
8	7.1139	7.0930	0.29
10	6.2788	6.5380	4.13
10	5.8751	6.5380	11.28
10	6.1761	6.5380	5.86
12	6.2304	5.9829	3.97
12	6.2788	5.9829	4.71
12	6.2553	5.9829	4.35

The analysis of observed and estimated values for D when the z value for B. stearothermophilus was determined from the plot of log D value vs temperature is given in Table 11. The % error describes the degree of variation between the observed and estimated values, which are from the best fit of log $y = A + Bx$.

In the regression analysis comparing the log number of colonies/ml of B. stearothermophilus (Table 8) vs time of heating, all of the temperatures had significant F values at the 5% and 1% level, which shows a high degree of correlation. The coefficient of multiple determination (R^2) for all four temperatures is also very high, such that the variation in number of survivors of B. stearothermophilus spores is associated with differences in time of heating. The data for 115 °C has the lowest R^2 value of 0.79, but this is probably because only three sets of data (3 processing times) were available for analysis.

TABLE 11

Comparison of the observed and estimated values for the determination of the z value (°C) from a plot of log D value vs temperature

Retort Temperature (°C)	Dependent Variable Values (Log D values) Observed	Estimated	% Error
110	1.4789	1.5341	3.73
115	1.3241	1.2229	7.64
121	0.8035	0.8495	5.72

The D values on the surface of potatoes, for B. stearothermophilus were found to be:

110°C	30.12
115°C	21.08
121°C	6.36
130°C	3.60.

The z value for B. stearothermophilus on the surface of potatoes heat treated in the retort was determined to be 16.07°C (28.9°F). This is considerably higher than the generally assumed z value of 10°C for spores and is over twice that listed by Gould and Hurst (1969, p. 577) of 7°C. Perhaps the z value being higher might be attributed to strain difference or to the possibility that the spores being present in the water-starch suspension on the surface of the potatoes are protected, such that more heat is required to do the same amount of damage.

The D values determined on the surface of potatoes for B. stearothermophilus compare favorably to the values reported in the literature, for example:

115°C	22.6 (Gould and Hurst, 1969)
115°C	25.0 (Briggs, 1966)
121°C	4 - 5 (Briggs, 1966)

but are sufficiently different in that the z value is much higher than that reported in the literature, probably because the D and z values are determined under different conditions or else because of strain difference.

F. Recovery of B. stearothermophilus from Inoculated Pouches

The method originally devised to determine that an adequate heat treatment was given to produce microbiologically stable potatoes involved processing pouches at different times at 121.1°C to determine what processing treatments would give a definite end-point of surviving micro-organisms. However, it was realized that an excessive number of spores/pouch (i.e. greater than 10^{12} or the equivalent of the harvest of spores from 1000 Roux bottles) would have to be used to get end-point survivors after a 12 D treatment. This excessive number of spores/pouch would mean a factory type production rather than production on a laboratory scale. Therefore, it was decided to test for the number of pouches spoiled with various heat treatments instead of determining the number of spores present in assessing the adequacy of sterilization treatment. However, before this method was tested it was considered necessary to examine the reproducibility of recovery of B. stearothermophilus spores from inoculated pouches.

Pouches of peeled potatoes were inoculated with a known number of spores and the contents were plated out using the same technique as for determining the natural level of contamination. Because the level of natural contamination (c. 3.9×10^3 spores/pouch) was so much smaller than the size of inoculum used (c. 5×10^5 spores and c. 5×10^7 spores/pouch) the number of colonies determined should approximate the size of inoculum used (see Table 12). To ascertain the reliability of the plating technique, and also to note the significant differences among the average viable spore counts, the Standard Error of the Mean was calculated. The formulae used were as follows:

Standard Deviation - $S = \sqrt{\frac{\Sigma X^2 - \frac{(\Sigma X)^2}{n}}{n - 1}}$

Standard Error of the Mean - $S_{\bar{x}} = \frac{S}{\sqrt{n}}$

The reproducibility obtained as noted by the averages approximating the size of inoculum used proves that conditions are not present in pouches for the duration that the spores were present, that would be deleterious to spores and prevent their germination. The reproducibility also justifies the technique used in plating (that is, the palpation by aid of the Vortex mixer) to determine the natural level of contamination.

The standard error of the mean is a measure of the reliability of the mean, thus for Run I the mean is $6.2 \times 10^5 \pm 12.9\%$ and for Run II the mean is $4.3 \times 10^7 \pm 18.6\%$. If further samples were drawn from the same population in 2/3 of the cases the means of the samples

TABLE 12

Reproducibility of plating technique used to determine colony counts of *B. stearothermophilus* in inoculated unprocessed pouches of potatoes

	Inoculum Size (spores/pouch) Run I c. 5×10^5	Run II c. 5×10^7
Counts Obtained (spores/pouch)	8.2×10^5	8.9×10^7
	8.0×10^5	6.4×10^7
	7.5×10^5	4.7×10^7
	5.5×10^5	7.9×10^6
	3.8×10^5	5.4×10^6
	3.9×10^5	
Average	6.2×10^5	4.3×10^7
$S_{\bar{x}}$	$\pm 0.8 \times 10^5$	$\pm 0.8 \times 10^7$

would be between 7.0×10^5 and 5.4×10^5 for Run I and 5.1×10^7 and 3.5×10^7 for Run II.

G. Determination of Adequate Treatment to the Center of the Potato for the Natural Level of Contamination

It is probable that the most common heat resistant micro-organism (anaerobe) that would be present on potatoes would be comparable to the test micro-organism P.A. 3679 (Cl. sporogenes). All the strains of P.A. 3679 readily available (i.e. American Type Culture Collection, Canada Packers, and the National Department of Health and Welfare) showed inadequate heat resistance or failed to produce a satisfactory crop of spores. As a consequence a more heat resistant micro-organism B. stearothermophilus (B7/202) was used. The D and z values of this micro-organism were determined and have already been given in section IV, E.

The D_{121} value for B. stearothermophilus on potatoes was found to be 6.36 minutes, which compares favorably to the value of 5, quoted by Briggs (1966). The D value of two different organisms can be compared providing they are determined at the same temperature. As there was little difference in the D_{121} value for B. stearothermophilus found in these experiments from the literature value then it is possible that the value given in the literature (Stumbo, Murphy and Cochran, 1950) of $D_{121} = 1.2$ minutes for P.A. 3679 would be similar to the D value determined on the surface of a potato, as the literature value of 1.2 was determined in various food products at 121.1°C (250°F) which may or may not be protective. The products varied, for example whole kernel corn, cooked fresh pork, white sauce and presterilized evaporated milk.

With the knowledge of the D value for the natural contaminating micro-organisms on the surface of the potato, the length of time for a 12 D treatment at 121°C on the surface of the potatoes can be calculated. For P.A. 3679 this would be 12 x 1.2 = 14.4 minutes. If a 12 D treatment is required at the center of the potato the process would have to be 14.4 + T, where T is the time necessary to reach processing temperature (121.1°C) at the center of the potato. With a T value of approximately 20 minutes, potatoes would have to be processed for 34.4 minutes, to give a 12 D treatment at the center. As a 1-3/4 in diameter potato is satisfactorily cooked in 16 minutes at 121°C, a treatment of 34 minutes would result in excessive overcooking and yield a product of unsatisfactory organoleptic quality.

Having determined that the maximum level of natural contamination on the surface is in the range of 10^3/potato, then the maximum at the center could also be about 10^3 if the potato had suffered internal damage and if no growth of micro-organisms had occurred. However, it is known that few potatoes do in fact suffer such damage (particularly small potatoes) and that the center of most potatoes is sterile (Thorpe and Atherton, 1972). Assuming that 1 potato per 1000 has micro-organisms at the center, and this estimate is probably excessive, then it would not be necessary to provide the same treatment at the center as on the surface of a potato. Stumbo (1965) suggests that processors find acceptable (but not desirable), a <u>potential</u> spoilage rate not greater than 1 container/thousand; this could of course result in an <u>actual</u> spoilage of no greater than 1 in 10,000, because most of the product will not be stored for the time necessary to induce maximum

spoilage, and it would be very easy for industry to include an adequate margin of safety by a slight modification in processing. Thus it may only be necessary to provide a treatment that will give less than 1 spore at the center of each potato of which only 1 in 1000 will be contaminated at the center. As the natural level of contamination is c. 4×10^3/pouch and the D_{121} value is 1.2 a treatment of 5 minutes at 121°C will give a treatment that would yield less than 1 spore at the center of each potato, or an F_o of 5. An F_o of 5 was estimated to be obtained at the center of each potato with the retort registering 121°C for a period of 20 minutes. An F_o of 8 was attained at the center of a potato after processing for 24 minutes.

In order to determine whether an F_o of 5 at 121°C at the potato center would be a satisfactory treatment with the natural level of contamination in pouches of potatoes the following experiment was done. Peeled and sized potatoes less than or equal to 1-3/4 in diameter were obtained from a local potato processor. Three to four potatoes were put in a pouch and vacuum sealed. Ten replicates were treated in the retort at the following times at 121°C: 17, 18, 19, 20, 21, 22, 23, 24 and 25 minutes. Two controls uninoculated and unprocessed were incubated at 30°C (see Prentice and Clegg, 1974), the optimum recovery temperature determined for mesophiles. After processing the pouches were dried, and placed in an incubator at 30°C for a sufficient length of time to see if the packs became blown. The two controls showed gas blowing after 3 - 5 days. After six months none of the processed pouches had shown gas blowing.

It would have been desirable to have used more than 90 pouches (at least 1000), and to have tested additional heat treatments less than 17 minutes until positive (blown) pouches occurred in the above experiment, to have practical proof of the margin of safety. However, as the controls were positive after 3 - 5 days and the 17 minute treatment was satisfactory, the recommended treatment of 20 minutes has a margin of safety of at least an F_o of 3 with the numbers of pouches used in this experiment. Had the number of pouches used in the experiment been increased ten fold (an additional 10 weeks' work) the possible margin of safety could have been reduced by 1 minute (if there were failures at the 17 minute processing time) and it was deemed with the facilities available that this was unwarranted.

The treatment (F_o of 5 at 121°C) that was given pouches of potatoes to determine whether the natural level of contamination would be destroyed turned out to be very satisfactory as none of the pouches blew after 6 months of storage at 30°C. The treatment deemed most likely to be satisfactory from a microbiologically and an organoleptic point of view was 20 minutes at 121°C, but even 17 minutes at 121°C appeared to give an adequate margin of safety on both considerations. However, because the F_o treatment received on the surface of potatoes would be much greater than $F_o = 5$ received at the center even at the lowest time, there would not be any surviving micro-organisms on the surface from the heat treatment that could cause pouches to blow. However, it is admitted that the potatoes used in this experiment were probably subjected to a more rigorous scrutiny than would be the case in commercial practice and therefore, the chances of including an

internally damaged potato would be small. This fact is borne in mind in the final conclusions.

V. SUMMARY AND CONCLUSIONS

To determine the satisfactory heat treatment required for any food it is necessary to know the natural level of contaminating micro-organisms, their heat resistance and the time-temperature treatment suitable for the food in question. The level of the natural contamination is influenced by the type and quality of the food, the method of preparation and handling and preparation of the raw food before processing. There is evidence that steam peeling of potatoes effectively lowers the natural contamination to 1 - 2 spores/potato (Thorpe, 1973) whereas results obtained in this laboratory showed that lye peeling is not as effective leaving c. 10^3 spores/potato. The time-temperature treatment found most satisfactory for small whole potatoes, in evacuated pouches, i.e. sufficiently cooked, was determined to be c. 16 minutes at 121.1°C in the retort. This information has to be used when determining which of a variety of time-temperature heat treatments should be used to produce a microbiologically stable product by destroying the natural level of contamination (4×10^3 spores/pouch) and at the same time result in a satisfactory product organoleptically.

The canning industry has accepted the use of P.A. 3679 as the test micro-organisms of choice, for heat resistance studies in food, because it is a heat resistant anaerobe which can survive some processing conditions and grow in a suitable substrate. This organism is more heat resistant than the pathogen *Clostridium botulinum*. Therefore in this work P.A. 3679 was first chosen for use in inoculation of

experimental packs. However, none of the strains tested were suitable as they were not sufficiently heat resistant. After trials with several anaerobic spore-formers it was eventually decided that it would be suitable to use an aerobic spore-former as a test organism providing its heat resistance was satisfactory and as long as growth of the inoculum on the surface of the food was not used as one of the criteria to determine satisfactory heat treatment, but merely tests for the presence of survivors. The spore-former chosen for further research was B. stearothermophilus. It would not have been possible to determine the satisfactory treatment to produce a microbiologically stable product with the natural level of contamination because with a desired processing treatment of 12 D an enormous number of samples would have been needed, such that only 1 in 10^{12} pouches would be unsterile. Therefore a search was made for a satisfactory heat treatment using levels of c. 5×10^7 spores/ml of B. stearothermophilus as an inoculum on the surface of potatoes in evacuated pouches so that definite survivor end-points could be achieved in heat treatment experiments and thermal death curves established.

From this information it was possible to determine the extinction treatment necessary for P.A. 3679 (the most heat resistant anaerobe thought to be associated with potatoes) after comparing the D values of the test micro-organism with D values in the literature available for P.A. 3679 at 121.1°C which was 1.2 minutes. But the process to give a 12 D treatment at the center of the potato would be 14.4 minutes plus the time necessary for the center to reach processing temperature (121.1°C), which was approximately 20 minutes. However,

14.4 + 20 or a process of 34 minutes would result in a grossly over-cooked product because only 16 minutes at 121 °C is necessary to produce a satisfactorily cooked potato.

Providing a potato has not suffered any internal damage the center is sterile (previous work in this laboratory confirmed this). Therefore a 12 D treatment to the center of the potato was considered to be unnecessary and only important at the surface. Estimates from workers in industry suggest that 1 in 1000 potatoes may be damaged at the center. This 1 in 1000 value falls within the accepted potential spoilage rate of not greater than 1 container/thousand (Stumbo, 1965). If a 12 D treatment is required at the surface then a processing time of c. 15 minutes should give satisfactory bacteriological results (12 x 1.2 = 14.4).

Proof that this treatment was satisfactory was determined by using short processing times at 121°C to get end points with the natural level of contamination of potatoes in evacuated pouches. However, for the processing times varying by minute intervals from 17 to 25 minutes no pouch failures were obtained after 6 months of storage, indicating a satisfactory margin of safety. The processing time of 20 minutes at 121°C is recommended because it included a margin of safety over the 17 minutes and if the critical D_{121} value for the natural contaminating micro-organism is 1.2, 5 minutes at 121°C or an F_o of 5 would yield less than 1 spore at the center of each potato and an F_o of 5 was estimated to be achieved within 20 minutes.

An alternative method for determining the satisfactory heat treatment for potatoes in laminated flexible pouches, would have been to

inoculate potatoes in a number of pouches with a standardized inoculum of the test micro-organism, comparable in number to that of the natural contaminants. Then the inoculated pouches would have been subjected to increasing periods of heating at 121.1°C in the retort, until a treatment achieved complete destruction of the inoculated spores. Then as the test micro-organism is more heat resistant than the naturally occurring contaminants, by a known factor (obtained by comparing their D_{121} values), a satisfactory processing treatment including an adequate margin of safety could have been determined.

Further recommendations that need to be carefully watched when processing a food in evacuated flexible pouches are:

1. the maximum temperature reached at the point of greatest temperature lag (center), which can be influenced by size of the potato, accounted for 93% of the variation in the F_o value;
2. pouches must be carefully handled so as to prevent wrinkle formation and occlusion of food particles. The type of product being tested will determine the amount of difficulty experienced with this problem, but various techniques and equipment have been developed which can control this;
3. the design of the rack should be such that pouches are quickly put and held in place and the convection currents are not hindered with a water cook process;
4. few difficulties were experienced with the water cook with 68.95 k Pa (10 lb/in^2) air overpressure used to process the flexible pouches, and the process can be speeded up by heating the water in the retort prior to introducing the pouches at the start of the process;

5. the optimum recovery temperature for the test organism should be determined, as this can alter the number of survivors by as much as 50% (Run II counts at 47 °C vs 54 °C);

6. although the D and z values determined for B. stearothermophilus on the surface of potatoes in evacuated pouches heated in a retort was different from values in the literature this was primarily due to strain differences rather than differences in conditions used, although the latter probably did influence the results obtained.

Finally, although the author has confidence in the work done, it is appreciated that the recommendations made on the pilot plant used, were based on a minimal amount of data. However, it was not the intention of this work to provide complete evidence on which a commercial operation could be based, because it will be appreciated that with this type of processing each plant layout must be tested separately so that the adequacy of the sterilization treatment used is assured. Rather, the purpose of the work has been to suggest a model system which could be used with any type of food and any type of naturally contaminating micro-organisms to determine an optimum, safe heat treatment which would produce a microbiologically stable product when the food was heat treated in a flexible package and stored at ambient temperature.

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APPENDIX 1

The equivalent time at the maximum temperature reached at the center of a potato with respect to F_o values

APPENDIX 1

The Equivalent Time at the Maximum Temperature Reached at the Center of a Potato with Respect to F_o Values

Maximum Temperature (T_M) Reached at the Center (°C)	Lethal Rate (L_i) for T_M	F_o	Equivalent Time (F_o:L_i) at T_M (min)
119.2	0.639	6.857	10.73
119.8	0.748	8.269	11.05
119.9	0.755	8.685	11.50
120.1	0.792	8.666	10.94
120.9	0.943	10.876	11.53
120.2	0.815	8.949	10.98
120.5	0.869	10.788	12.41
121.0	1.000	13.598	13.60
120.5	0.869	10.574	12.17
119.9	0.757	8.988	11.87
121.0	1.000	10.505	10.50
118.0	0.489	3.200	6.54
118.6	0.561	3.655	6.52
118.0	0.489	3.207	6.56
119.0	0.615	4.277	6.95

APPENDIX 2

Examples of discrepancies in colony count from different dilutions from the same inoculum (results from the gradient temperature incubator experiment)

APPENDIX 2

Examples of Discrepancies in Colony Count from Different Dilutions from the Same Inoculum (results from the gradient temperature incubator experiment)

Temperature of Incubation (°C)	Unheated Spores (counts/pouch from duplicate plates) Dilution 10^{-5} a	10^{-5} b	10^{-6} a	10^{-6} b	Average* (x 10^6)
44	62	88	18	17	12.5
47	79	82	19	36	17.8
51	107	84	21	21	15.0
54	116	108	20	50	23.1
57	95	75	(11)	(12)	8.5
61	36	48	(14)	(10)	4.2
65	17	(3)	(11)	(13)	1.7

Temperature of Incubation (°C)	Heated Spores Run I (counts/pouch from duplicate plates) Dilution 10^{-1} a	10^{-1} b	10^{-2} a	10^{-2} b	Average* (x 10^2)
44	35	29	17	-	10.1
47	45	47	19	19	12.0
51	46	47	(10)	(6)	4.6
54	19	21	29	21	14.0
57	21	21	16	26	11.6
61	86	133	17	(9)	11.0
65	(10)	(5)	(13)	(6)	0.75

Temperature of Incubation (°C)	Heated Spores Run II (counts/pouch from duplicate plates) Dilution 10^{-2} a	10^{-2} b	10^{-3} a	10^{-3} b	Average* (x 10^3)
44	107	114	19	-	15.0
47	178	110	21	-	17.7
51	128	59	(11)	-	9.3
54	87	64	(6)	-	7.5
57	117	126	46	-	29.1
61	107	91	20	-	15.0
65	112	91	33	-	22.0

* the average must be divided by 0.2 to get the same figures as in Table 7

() figures in parentheses not included in averages as below accepted range of 15 - 150 counts/roll tube

APPENDIX 3

Colony counts used for the determination of the decimal reduction time

APPENDIX 3

Colony Counts used for the Determination of Decimal Reduction Time (colony counts are averages of triplicate plates from triplicate pouches per run)

Processing Time (min)	Processing Temperature (°C)	Colony Counts (spores/ml)	Logarithms
40	110	1.4×10^9	9.1461
		1.9×10^9	9.2788
		1.8×10^9	9.2553
60	110	1.4×10^8	8.1461
		1.9×10^8	8.2788
		2.1×10^8	8.3222
100	110	1.1×10^7	7.0414
		1.4×10^7	7.1461
		2.3×10^7	7.3617
120	110	2.4×10^6	6.3802
		3.0×10^6	6.4771
		2.9×10^6	6.4624
20	115	1.7×10^8	8.2355
		1.1×10^8	8.0414
		1.8×10^8	8.2553
40	115	1.7×10^6	6.2227
		1.4×10^6	6.1335
		1.5×10^6	6.1732
68	115	7.5×10^5	5.8751
		5.8×10^5	5.7634
		5.4×10^5	5.7324
5	121	8.9×10^7	7.9494
		6.4×10^7	7.8062
		4.7×10^7	7.6721
10	121	7.9×10^6	6.8976
		5.4×10^6	6.7324
13	121	3.5×10^6	6.5441
		4.6×10^6	6.6628
		4.6×10^6	6.6628
15	121	1.4×10^6	6.1614
		1.5×10^6	6.1818
		1.5×10^6	6.1703
4	130	1.9×10^8	8.2788
		2.0×10^8	8.3010
8	130	1.6×10^7	7.2041
		1.8×10^7	7.2553
		1.3×10^7	7.1139
10	130	1.9×10^6	6.2788
		7.5×10^5	5.8751
		1.5×10^6	6.1761
12	130	1.7×10^6	6.2304
		1.9×10^6	6.2788
		1.8×10^6	6.2553

APPENDIX 4

The computer programs used in the regression analyses

APPENDIX 4

The computer programs used in the regression analyses are listed and given in full here.

Reghow

Reshow

Streghow

RRP (Regression-Residuals-Plot) including the sub programs used for this program:

Inv

Sub

Rout

Tout

Reg

Res

REGHOW

SIMPLE AND MULTIPLE REGRESSION
T←V REG X
ENTERED:24/05/68

X IS A MATRIX OF OBSERVATIONS, WHERE THE COLUMNS CORRESPOND TO VARIATES AND THE ROWS TO OBSERVATIONS. V IS A VECTOR OF POSITIVE INTEGERS. T IS A MATRIX OF 5 COLUMNS. AS AN EXAMPLE OF THE OUTPUT, LET X HAVE 6 COLUMNS, AND LET V=(3,5,1,4). THEN T GIVES THE RESULTS OF THE BEST LEAST-SQUARES FIT OF THE FUNCTION

$$X4=A+B\times X3+C\times X5+D\times X1$$

IN THE FOLLOWING FORMAT:

ROW1: 4, A, 0, 0, 0
ROW2: 3, B, ST ERROR OF B, T-VALUE, 0
ROW3: 5, C, ST ERROR OF C, T-VALUE, 0
ROW4: 1, D, ST ERROR OF D, T-VALUE, 0
ROW5: 0, DF FOR REGRESSION, SUM OF SQUARES, MEAN SQUARE, F-VALUE
ROW6: 0, DF FOR ERROR, SUM OF SQUARES, MEAN SQUARE, 0
ROW7: 0, DF FOR TOTAL, SUM OF SQUARES, ST ERROR OF ESTIMATE, SQUARE OF MULTIPLE CORR COEFF

REQUIRES INV.

```
      ∇REG[⎕]∇
    ∇ T←V REG X;N;U;M;R;Q;S;B
[1]   T←((3+⍴V),5)⍴0
[2]   T[⍳⍴V;1]←V[⍋V],¯1↓V
[3]   Q←(⌹R)+.×R←U-(⍴U)⍴M←(+/[1] U←X[;V])÷N←(⍴X)[1]
[4]   T[3+⍴V;3]←Q[⍋V;⍴V]
[5]   Q←¯1 0 ↓Q
[6]   B←(-M+.×B,¯1),B←,(S←⌹ 0 ¯1 ↓Q)+.×Q[;⍋V]
[7]   T[⍳⍴V;2]←B
[8]   T[1+⍴V;3]←(1↓B)+.×Q[;⍋V]
[9]   T[(⍴V)+⍳3;2]←((⍴V)-1),(N-⍴V),N-1
[10]  T[2+⍴V;3]←-/T[(3+⍴V),1+⍴V;3]
[11]  T[(⍴V)+⍳2;4]←T[(⍴V)+⍳2;3]÷T[(⍴V)+⍳2;2]
[12]  T[1+⍳(⍴V)-1;3]←(T[2+⍴V;4]×+/S×(⍳(⍴V)-1)∘.=⍳(⍴V)-1)*0.5
[13]  T[1+⍳(⍴V)-1;4]←T[1+⍳(⍴V)-1;2]÷T[1+⍳(⍴V)-1;3]
[14]  T[3+⍴V;4]←T[2+⍴V;4]*0.5
[15]  T[1+⍴V;5]←T[1+⍴V;4]÷T[2+⍴V;4]
[16]  T[3+⍴V;5]←T[1+⍴V;3]÷T[3+⍴V;3]
    ∇
```

```
      RESHOW

          RESIDUALS
          R←T RES X
          ENTERED:24/05/68

   X IS THE MATRIX OF OBSERVATIONS DEFINED FOR REG, AND T IS
THE RESULT OF USING REG WITH SOME VECTOR V. R IS A MATRIX,
WITH 4 COLUMNS AND THE NUMBER OF ROWS EQUAL TO THE NUMBER
OF ROWS IN X, WHICH GIVES THE FOLLOWING RESULTS OF FITTING
THE REGRESSION SPECIFIED BY X AND V:
          COL 1:   1,2,...
          COL 2:   OBSERVED VALUES OF DEPENDENT VARIABLE
          COL 3:   ESTIMATED VALUES OF DEPENDENT VARIABLE
          COL 4:   RESIDUALS


      ∇RES[⎕]∇
    ∇ R←T RES X;V;O;E
[1]   V←T[1+⍳¯4+⍴T[;1];1]
[2]   R←(O←X[;T[1;1]])-E←T[1;2]+T[1+⍳⍴V;2]+.×⍉X[;V]
[3]   R←⍉(4,(⍴X)[1])⍴(⍳(⍴X)[1]),O,E,R
    ∇
```

STREGHOW

STEPWISE REGRESSION
T←V STREG X
ENTERED:24/05/68

THIS PROGRAM IS IDENTICAL IN FUNCTION TO THE SIMPLE AND MULTIPLE REGRESSION PROGRAM REG EXCEPT THAT THE INDEPENDENT VARIABLES ARE ENTERED INTO THE REGRESSION FUNCTION IN THE STEPWISE ORDER.

THE VECTOR V IS IDENTICAL TO THE VECTOR V IN REG EXCEPT THAT THE INDEPENDENT VARIABLES MAY BE SPECIFIED IN V IN ANY ORDER. THE FORMAT OF THE MATRIX OF RESULTS T IS IDENTICAL TO THE MATRIX T OF REG EXCEPT THAT THE PROPORTION OF THE VARIATION OF THE DEPENDENT VARIABLE ACCOUNTED FOR BY EACH INDEPENDENT VARIABLE IS GIVEN IN THE FIFTH COLUMN OF T IN THE ROWS CONTAINING THE REGRESSION COEFFICIENTS, STANDARD ERRORS AND T-VALUES FOR THE INDEPENDENT VARIABLES.

REQUIRES REG, CM.

```
      ∇STREG[⎕]∇
    ∇ T←V STREG X;R;S;M;I;J;K;A;B
[1]   R←CM X[;V]
[2]   S←⍳0
[3]   S←S,M,V[K←(|,R[I;J])⍳M←⌈/|,R[I←⍳(J-1);J←⍴V]]
[4]   B←(R∘.×B←1-(A←(K←K≠⍳J)/R[;K])*2)*0.5
[5]   V←K/V
[6]   →(1<(⍴R←((K/[1] K/R)-A∘.×A)÷B)⌈1])/3
[7]   T←(((0=2|⍳⍴S)/S),V) REG X
[8]   R←((1=2|⍳⍴S)/S)*2
[9]   S←(⍳0),0
[10]  S←S,R[⍴S]×1-+/S
[11]  →((⍴S)≤⍴R)/10
[12]  T[⍳⍴S;5]←S
    ∇
```

The RRP (Regresson-Residuals-Plot) program includ-
the sub programs used in this program

```
      ∇RRP[□]∇
    ∇ RRP;D;VO;T;R;N
[1]   ⍝PROGRAM COMBINATION AND MODIFICATIONS BY L. STEELE
[2]   ⍝LATEST ALTERATION: NOVEMBER/75
[3]    '
       REGRESSION → RESIDUALS → PLOT
       '
[4]   L1:'ENTER DATA:',VO←''
[5]    →□LC IF YES,0⍴□←'MORE? (YES OR NO)',0⍴VO←VO,□
[6]    →L2 IF~2|⍴N←VO
[7]    'ODD NUMBER OF ENTRIES!'
[8]    →L1,0⍴□←'--WHAT YOU DID IS STORED AS ''N'''
[9]   L2:'
       REGRESSION DEGREE? (1,2,3,4, OR 5)'
[10]   →L3 IF(D∈1+⍳5)∧1=+/⍴,D←1+□
[11]   →L2,0⍴□←'DEGREE MUST BE ONE OF→',▼⍳5
[12]  L3:VO←1⌽((0.5×⍴N),2)⍴N
[13]   →L4 IF D≤2
[14]   VO←VO,VO[;2]∘.*1+⍳D
[15]  L4:T←(1⌽⍳D) REG VO
[16]   '
       **REGRESSION.  BEST LEAST SQUARES FIT OF  Y = ';((D=
       1)/'A'),(8×D-1)↑'A + B×X + C×X*2 + D×X*3 + E×X*4 + F
       ×X*5
       '
[17]   TOUT
[18]   R←T RES VO
[19]   '
       **RESIDUALS:
       '
[20]   ROUT
[21]   '

       IS A PLOT DESIRED?'
[22]   →L5 IF NO
[23]   40 80 PLOT R[;2] AND R[;3] VS,VO[;2]
[24]   '
            (∘ OBSERVED; * ESTIMATED)'
[25]  L5:'
       REPEAT WITH A DIFFERENT DEGREE?'
[26]   →L2 IF YES
    ∇
```

```
      ∇INV[⎕]∇
    ∇ RB←INV RA;RK;RS;RP;RI
[1]   'NEWER VERSIONS OF APL USE PRIMITIVE OPERATOR ⌹ FORM
      ED BY OVERSTRIKING ⌹ AND ÷'
[2]   'AND CONSEQUENTLY ALL FUNCTIONS IN THIS PACKAGE USE
      THE OPERATOR INSTEAD OF THE'
[3]   'ORIGINAL INVERSE FUNCTION WHICH IS GIVEN HERE'
[4]   →((2=ρρRA)∧=/ρRA)ρL2
[5]  L1:'NO INVERSE!'
[6]   →~RB←1
[7]  L2:RK←⌊/ρRA
[8]   RS←RK
[9]   RP←⍳RK
[10]  RA←RA[;(⍳RS),1]
[11] L3:RA[;1+RS]←(⍳RS)≤1
[12]  RI←(|RA[⍳RK;1])⍳⌈/|RA[⍳RK;1]
[13]  RP[1,RI]←RP[RI,1]
[14]  RA[1,RI;⍳RS]←RA[RI,1;⍳RS]
[15]  →(1E¯30>|RA[1;1])ρL1
[16]  RA[1;]←RA[1;]÷RA[1;1]
[17]  RA←RA-((~(⍳RS)≤1)×RA[;1])∘.×RA[1;]
[18]  RA←RA[1+RS|⍳RS;(1+⍳RS),1]
[19]  RP←RP[1+RS|⍳RS]
[20]  →(0<RK←RK-1)/L3
[21]  RB←RA[;RP⍳⍳RS]
    ∇


      ∇SUB[⎕]∇
    ∇ Z←SUB
[1]   Z←M[N],', ST ERROR OF ',M[N],', T-VALUE:'
    ∇


      ∇ROUT[⎕]∇
    ∇ ROUT
[1]   '          DEPENDENT VARIABLE VALUES'
[2]   '      ..................  ..................  ..................'
[3]   '
[4]   (4 0 ,6ρ 15 5)▼R
    ∇
```

```
      ∇TOUT[□]∇
    ∇ TOUT;M;N
[1]   0ρM←'ABCDEF'
[2]   'A  →  ';T[N←1;2];'
      -----'
[3]  LA:→LB IF D<N←N+1
[4]   SUB
[5]   '      ';T[N;1+ι3]
[6]   →LA,0ρ□←'-----'
[7]  LB:'DF FOR REGRESSION, SUM OF SQUARES, MEAN SQUARE, F
      -VALUE:'
[8]   '      ';T[N;1+ι4]
[9]   'DF FOR ERROR, SUM OF SQUARES, MEAN SQUARE:',0ρ□←'--
      ---'
[10]  '      ';T[N+1;1+ι3]
[11]  'DF FOR TOTAL, SUM OF SQUARES, ST ERROR OF ESTIMATE,
       SQUARE OF MULTIPLE CORR COEFF:',0ρ□←'-----'
[12]  '      ';T[N+2;1+ι4]
    ∇


      ∇REG[□]∇
    ∇ T←V REG X;N;U;M;R;Q;S;B
[1]   T←((3+ρV),5)ρ0
[2]   T[ιρV;1]←V[ρV],¯1↓V
[3]   Q←(⌹R)+.×R←U-(ρU)ρM←(+/[1] U←X[;V])÷N←(ρX)[1]
[4]   T[3+ρV;3]←Q[ρV;ρV]
[5]   Q←¯1 0↓Q
[6]   B←(-M+.×B,¯1),B←,(S←⌹0 ¯1↓Q)+.×Q[;ρV]
[7]   T[ιρV;2]←B
[8]   T[1+ρV;3]←(1↓B)+.×Q[;ρV]
[9]   T[(ρV)+ι3;2]←((ρV)-1),(N-ρV),N-1
[10]  T[2+ρV;3]←-/T[(3+ρV),1+ρV;3]
[11]  T[(ρV)+ι2;4]←T[(ρV)+ι2;3]÷T[(ρV)+ι2;2]
[12]  T[1+ι(ρV)-1;3]←(T[2+ρV;4]×+/S×(ι(ρV)-1)∘.=ι(ρV)-1)*
      0.5
[13]  T[1+ι(ρV)-1;4]←T[1+ι(ρV)-1;2]÷T[1+ι(ρV)-1;3]
[14]  T[3+ρV;4]←T[2+ρV;4]*0.5
[15]  T[1+ρV;5]←T[1+ρV;4]÷T[2+ρV;4]
[16]  T[3+ρV;5]←T[1+ρV;3]÷T[3+ρV;3]
    ∇


      ∇RES[□]∇
    ∇ R←T RES X;V;O;E
[1]   V←T[1+ι¯4+ρT[;1];1]
[2]   R←(O←X[;T[1;1]])-E←T[1;2]+T[1+ιρV;2]+.×⌹X[;V]
[3]   R←⌹(4,(ρX)[1])ρ(ι(ρX)[1]),O,E,R
    ∇
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